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Studies on Barbiturates XXIV

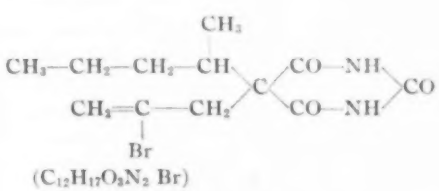
Pharmacology of Secondary Amyl-Beta-Bromallyl Barbituric Acid*

By Lloyd W. Hazleton, Theodore Koppanyi and Charles R. Linegar†

This study is the survey of the pharmacological actions and toxic properties of a relatively new barbiturate; 5-sec. amyl-5-beta-bromallyl barbituric acid, named "Sigmodal" by its manufacturers, Messrs. Riedel-de Haen, Inc.

CHEMISTRY

This compound is a white crystalline powder of bitter taste with the following structural formula:



$(C_{12}H_{17}O_3N_2\ Br)$

It is difficultly soluble in water and chloroform, soluble in ether and alcohol, and melts at 161° to 163° C. The sodium salt is soluble in water, more difficultly soluble in alcohol and practically insoluble in ether. The aqueous solution is unstable and should not be used after a few days of standing. A 10 per cent solution of sodium sec. amyl bromallyl barbiturate in an aqueous vehicle, each 100 cc. of which contains 10 Gm. of glycerine, 10 Gm. of antipyrine and 10 cc. of alcohol as stabilizing agents, is recommended by the manufacturers for rectal administration.

Sigmodal gives in chloroform solution the characteristic blue color in the Koppanyi test for barbiturates (1), (2), (3), (4). There is an apparent decrease of sensitivity to the test owing to the high molecular weight of the compound (5), the color being about 60 per cent that of barbital. The readings for the test are made as for barbital and then corrected by multiplying the results by 1.7.

The technique used in estimating sigmodal in the urine, etc., is the same as that for barbital except that ether is used for extraction. If chloroform is used for extraction, at least three extractions should be made because of the low solubility of this compound in chloroform. The ether or chloroform extracts are evaporated to dryness and the residues taken up in chloroform for the colorimetric estimations. It is possible to make a 0.2 per cent solution of the acid in chloroform which is sufficient for the needs of the test.

EXPERIMENTAL

Sigmodal was used for intravenous injections in a 2 per cent aqueous solution of the sodium salt, and

* This substance was prepared in the laboratories of Riedel-de Haen, Inc., and a grant by this company partially defrayed the expense of this study.

† From the Department of Pharmacology and Materia Medica Georgetown University School of Medicine, Washington, D. C.

occasionally, for prolonged intravenous infusions in a 0.2 per cent solution. The 10 per cent solution of the sodium salt in water, with 10 per cent each of glycerine, antipyrine and alcohol as supplied by the manufacturers was used for rectal administration.

TOXICITY

The average (50 per cent) fatal dose was determined intravenously in rabbits and dogs, and rectally in rabbits. The results are shown in Tables I, II and III.

Table I.—Results of Intravenous Administration of Sigmodal Sodium in Rabbits

Dose Mg./Kg.	Number of Animals			Recovery[a] Time			
	Total	Lived	Died	Minimum Minutes	Maximum Minutes	Average Minutes	Time of Death Minutes
10	12	12	0	...	...	0	.....
15[b]	6	6	0	...	...	30	.....
20	3	3	0	53	80	64	.....
25	5	5	0	45	72	67	.....
30	7	7	0	64	122	88	.....
40	11	6	5	105	125	118	3 to 15
45	5	0	5	...	...	...	2 to 8

[a] Ability to maintain upright posture unassisted.
[b] Loss of motor coördination; righting reflex absent in four animals.

Table II.—Results of Rectal Administration of Sigmodal Sodium in Rabbits

Dose Mg./Kg.	Number of Animals			Average Recovery Time Minutes	Time of Death Minutes
	Total	Lived	Died		
50[a]	7	7	0	98	.....
60	10	9	1	158	40
80	2	2	0	240	.....
90	5	3	2	294	11 and 34
100	2	0	2	...	22 and 36

[a] No narcosis in two animals.

Table III.—Results of Intravenous Administration of Sigmodal Sodium in Dogs

Dose Mg./Kg.	Number of Animals			Average Recovery Time Hours	Time of Death Minutes	Remarks
	Total	Lived	Died			
20	2	2	0	1.8	.....	Righting reflexes present, incomplete relaxation, poor analgesia
25	2	2	0	...	.....	Good anesthesia, surgery performed
30	6	6	0	5	.....	Good anesthesia, surgery performed
35	2	1	1	About 6	3	
40	3	0	3	...	Almost immediate respiratory stoppage	.....

In rabbits the 50 per cent intravenous fatal dose is approximately 40 mg. per Kg.,[1] whereas all animals receiving 45 mg. died. The 50 per cent rectal fatal dose in rabbits is about 90 mg., *i. e.*, slightly more than twice the intravenous dose, while 100 mg. produced 100 per cent fatalities. In the dog the average intravenous fatal dose is about 35 mg. A dose of 30 mg. failed to kill any of six animals. The data on the rectal administration of sigmodal to dogs are too incomplete to warrant a general statement. The cause of death is always arrest of respiration, the heart continuing to beat vigorously for several minutes after respiration has ceased. In rabbits the average intravenous fatal dose caused death in about eight minutes, and the rectal fatal dose in about twenty minutes following administration of sigmodal sodium. In dogs death following intravenous doses occurs more rapidly.

[1] All doses are expressed in terms of mg. of sigmodal sodium per Kg. of body weight. To avoid repetition the words "per kilogram of body weight" will hereafter be omitted.

EXCRETION

The excretion of sigmodal in the urine was determined in rabbits and dogs. Five rabbits (No. 7, 8, 9, 10 and 11 of Table IV) showed during the first six hours the following percentage excretion in the urine: 7, 17, 3.8, 4.5 and 5.6 per cent respectively.

In two dogs a single dose of 30 mg. of sigmodal sodium was given intravenously and the barbiturate content of the urine was determined during a period of 48 hours. Only traces of the drug were excreted during the second 24 hour period. In the first dog a

total excretion of 20 per cent and in the second, of 21.8 per cent was observed. These figures represent the results obtained by using the Koppanyi colorimetric test for barbiturates, modified as described in the section on Chemistry.

ESSENTIAL ELIMINATION

The persistence of action of sigmodal was studied in rabbits by two different methods:

In the first method fractions of the intravenous 50 per cent fatal dose were injected at half-hourly and hourly intervals until death occurred. The difference between the fatal dose determined in this way and that determined by a single injection was taken to represent approximately the amount of drug essentially eliminated during the period between the administration of the first fraction and the death of the animal.

The second method is a new one which may be described briefly as follows: The maximum dose which permitted the animals to maintain normal posture unsupported and to retain approximately normal motor activity was determined. This dose for sigmodal sodium was found to be about 10 mg. intravenously, which is 25 per cent of the 50 per cent intravenous fatal dose as determined by a single injection. After ascertaining the average sleeping time of all survivors from the average fatal dose, *i. e.*, until they regained the stage in which normal body posture was again maintained, the essential elimination of the compound was computed by the following formula:

$$\frac{\text{50 per cent fatal dose—maximum dose of hypnotic permitting normal motor activity}}{\text{sleeping time (hours)}} = \text{essential elimination in milligrams (per kilogram) per hour.}$$

Table IV shows the results arrived at by the first method. Five rabbits were given 15 mg. of sigmodal sodium intravenously every half-hour, five rabbits 10 mg. every half-hour, and six rabbits 10 mg. every hour. Two rabbits showing little if any elimination, presumably exceptional or sick animals, were not included in the table. The animals receiving 10 mg. per hour showed no visible signs of accumulation, and after seven doses they were apparently in the same condition as after the first dose, therefore, they eliminated more than 25 per cent of the fatal dose per hour. Of the rabbits receiving 10 mg. every half-hour, only one died after thirteen doses; the others showed deep 4+ anesthesia but were found to be completely recovered the next morning. Therefore, they eliminated 225 per cent of the average fatal dose or more during a period of about six hours, or more than 37 per cent per hour. The rabbits receiving 15 mg. every half-hour eliminated on the average a little over one fatal dose during a period of two hours and twenty-two minutes or 47 per cent per hour. All these re-

Table IV.—The Essential Elimination of Sigmodal Sodium after Repeated Intravenous Administration of Fractions of the Fifty Per Cent Fatal Dose in Rabbits

Rabbit Number	Single Dose, Mg./Kg.	Number of Doses	Interval between Doses, Minutes	Results[a]	Total Dose Given, Mg./Kg.	Total Dose in Percentage of 50 Per Cent Fatal Dose, Per Cent	Duration of Experiment, Minutes	Percentage Essential Elimination of 50 Per Cent Fatal Dose, Per Cent
1	10	7	60	No narcosis[b]	70	175	...	.....
2	10	7	60	No narcosis[b]	70	175	...	.....
3	10	7	60	No narcosis[b]	70	175	...	.....
4	10	7	60	No narcosis[b]	70	175	...	.....
5	10	7	60	No narcosis[b]	70	175	...	.....
6	10	7	60	No narcosis[b]	70	175	...	.....
	Average							> 25 per hour
7	10	13	30	R	130	325	...	> 225
8	10	13	30	D(7)	130	325	367	225
9	10	13	30	R	130	325	...	> 225
10	10	13	30	R	130	325	...	> 225
11	10	13	30	R	130	325	...	> 225
	Average							> 37 per hour
12	15	4	30	D(7)	60	150	97	50
13	15	7	30	D(5)	105	263	178	163
14	15	6	30	D(10)	90	225	160	125
15	15	7	30	D(2)	105	263	179	163
16	15	4	30	D(7)	60	150	97	50
	Average						142	110
								47 per hour

[a] D = Death; R = Recovery; Interval in minutes between last dose and death in parenthesis.
[b] No determinable accumulation from hourly doses of 10 mg.

sults seem to indicate that rabbits are capable of eliminating about 45 per cent of the fatal dose, or 18 mg. (per Kg.) per hour.

In determining the essential elimination by the second method, use is made of certain data shown in Table I. The 50 per cent fatal dose was 40 mg., the average sleeping time of the survivors from this dose was 118 minutes (2 hours), and the maximum dose not interfering with body posture and motor activity was 10 mg. Thus the essential elimination may be determined by substituting in the above formula: $\frac{40 - 10}{2} = 15$ mg. (per Kg.) per hour, a figure which agrees fairly closely with the figure obtained by the first method.

ACTION ON THE CENTRAL NERVOUS SYSTEM

Sigmodal sodium, in appropriate doses, produces incoördination, sleep, motor paralysis and anesthesia. Complete sensory paralysis is seen only in dogs with doses from 30 to 35 mg. In rabbits, even amounts approaching the fatal dose do not produce

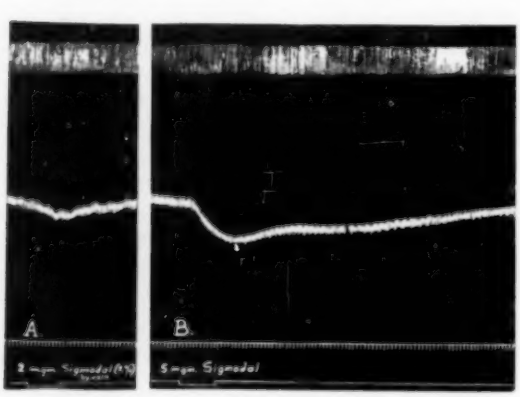


Fig. 1.—Dog. Male, Weight 6.7 Kg. Ether Anesthesia. Upper line respiration, second line blood pressure from the common carotid artery, third line base line. Time = 1.7 seconds. A. Intravenous injection of 2 mg. sigmodal sodium; B. Intravenous injection of 5 mg. sigmodal sodium. Note absence of effect on respiration.

corneal anesthesia or loss of response when the tail is pinched, or when intravenous injections are made into the ear; also the superficial tendon reflexes are retained. In rabbits the effects of 10, 15, 20, 25, 30, 40 and 45 mg. given intravenously and 40, 50, 80, 90 and 100 mg. administered rectally were studied. The rabbits receiving the 10 mg. intravenous dose retained their normal posture and were able to hop around with no, or only a slight, motor incoördination. With 15 mg. doses the righting reflexes were retained but motor coördination was lost. With 20 mg. doses the animals lost their righting reflexes and assumed a lateral position, but the muscular relaxation was very incomplete and the analgesia poor. With doses of 25 mg. or more, most of the animals showed complete muscular relaxation (all animals with 30 mg. or more but not all with 25 mg.), fair analgesia, frequently nystagmus, a state corresponding to Schoen's 4+ stage of anesthesia (6). Upon rectal administration the effective doses were much higher; with 50 mg. some animals retained their righting reflexes while with 60 mg. all animals showed 4+ anesthesia.

Dogs show true surgical, third stage anesthesia with loss of corneal reflex, complete analgesia and muscular relaxation. Several surgical operations were performed on dogs receiving 25 to 35 mg. of sigmodal sodium intravenously. The results from rectal administration of sigmodal sodium in dogs were not as uniform as in rabbits; in some animals 40, 50, 80 and 100 mg. produced about the same degree of depression (sleep, but not true anesthesia) while 150 mg. produced surgical anesthesia, and 250 mg. caused death in about five hours.

The duration of narcosis is relatively brief in rabbits; in dogs after comparable doses, it is at least twice as long. Sigmodal sodium is definitely a short-acting barbiturate.

The onset of action after intravenous injection is immediate, the animals often relaxing before the end of the injection. Upon rectal administration the onset of action in rabbits is very rapid, motor incoördination becoming evident in 2 to 8 minutes, while with suitable doses 4+ anesthesia occurs in less than 15 minutes.

The peripheral nervous system is apparently little affected by this drug; there is no paralysis of the cardiac vagus as determined by faradic stimulation even with fatal doses, and the chorda tympani likewise remains active. The cardiac and vasomotor responses to autonomic drugs are normal.

HEART, CIRCULATION AND RESPIRATION

In small or in moderate doses, sigmodal sodium had no effect on the rate or rhythmicity of the heart. With large doses a moderately increased pulse pressure was observed with no appreciable decrease in the rate.

Upon intravenous injection sigmodal sodium produced vasodilation and fall of blood pressure (Fig. 1).

These vasodepressor effects are similar to those produced by comparable doses of other barbiturates. The magnitude of the vasodilator effects depends upon the rate of injection, the size of the dose and the concentration of the solution. Slow intravenous infusion of the 0.2 per cent sigmodal sodium solution produced little or no change in blood pressure if the dose did not exceed 10 mg. Larger doses of sigmodal sodium (35 mg. or more) given by slow intravenous infusion produced a gradual fall of blood pressure terminating in circulatory collapse which occurred only after respiration had ceased for some time. Narcotic doses given intravenously or rectally for the purposes of acute laboratory experiments do not produce abnormally low blood pres-

sures. Dogs receiving 25 to 35 mg. of sigmodal sodium intravenously have an initial blood pressure of the same magnitude, 120 to 170 mm. Hg, as seen under other types of anesthesia; another dog receiving an anesthetic dose rectally had a blood pressure of 164 mm. Hg; and a rabbit receiving an anesthetic dose rectally had a blood pressure of 116 mm. Hg. Continuous administration of ether through the tracheal cannula, when the animals are under full effects of sigmodal sodium, results in a slight lowering of the blood pressure. Unless lethal or nearly lethal quantities of sigmodal are present in the animal the recoveries from the vasodepressor effects are fairly rapid.

This drug produced a slowing and decreased amplitude of the respiratory movements (Fig. 2), the slowing and decrease being proportional to the dose. With fatal doses the respiration may assume either Cheyne-Stokes rhythm or a form which is rather peculiar and perhaps characteristic of this drug. This latter type of respiration is shown in Fig. 2.

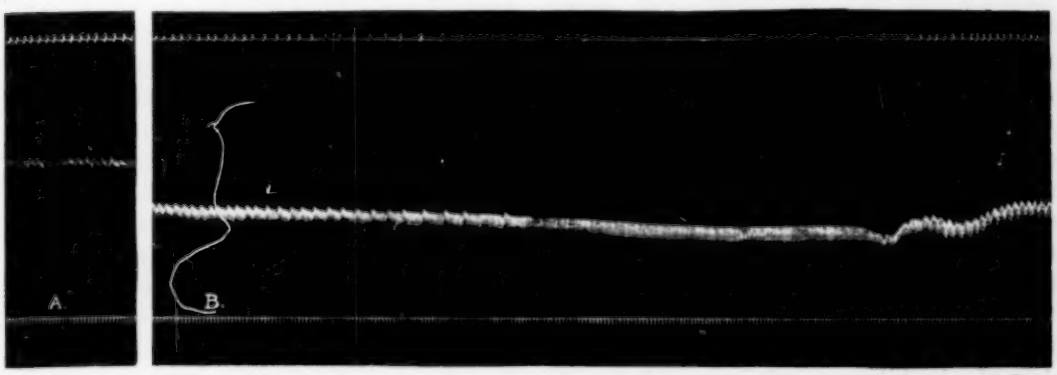


Fig. 2.—Dog, Male, Weight, 8.0 Kg., 250 mg. Sigmodal Sodium by rectum. A. One hour after administration. B. Three hours after administration. Upper line respiration, second line blood pressure, lower line base line. Time = 1.7 seconds. Animal subsequently died.

After a fairly long normal period, the respiratory rate slows gradually and apnœa occurs for a period of about three minutes during which time there is a slight fall in blood pressure. At the end of this period respiration is resumed at the previous rate and the blood pressure recovers. This phenomenon may be observed several times before death occurs from the apnoea. Death from sigmodal sodium is always a typical respiratory death.

Metrazol increases the rate and depth of respiration following respiratory depression by toxic doses of sigmodal sodium.

ANTAGONISMS

In order to study the anti-convulsant and life-saving effects of sigmodal in poisoning by convulsants, the following experiments were performed:

(*a*) Three rabbits received 30 mg. of sigmodal sodium by vein, and ten minutes later the first received 0.36, the second 0.72 and the third 1.44 mg. (per Kg.) of strychnine sulfate (*i. e.*, one, two and four average fatal doses) by vein. There were immediate convulsions in all three animals, but they recurred infrequently and, especially in the first two animals, they were not of the violent type. All three rabbits recovered, but the recovery time was prolonged in all. The last rabbit receiving four fatal doses of strychnine developed a persistent spastic paralysis of the hind limbs.

(*b*) Four hundred mg. (per Kg.) of antipyrine, administered by vein produces tonic and clonic convulsions in rabbits. In two rabbits 30 mg. of sigmodal sodium prevented the appearance of convulsions from this dose of antipyrine. The recovery time of these animals was about the same as that of the control animals receiving only 30 mg. of sigmodal sodium. The administration of antipyrine did not increase the depth of depression or analgesia in the sigmodal sodium-treated rabbits.

(*c*) Two rabbits, receiving 25 mg. each and one rabbit receiving 30 mg. of sigmodal sodium intravenously, were given eight minutes later 50 mg. of metrazol (per Kg.) by vein. Immediate return of the righting reflexes followed the injection of metrazol and final recoveries were observed twelve, thirty-four and thirty-three minutes later. (cf. Table I for normal recoveries.)

DISCUSSION

Sigmodal can apparently be extracted from the urine by methods ordinarily used for barbiturates and estimated colorimetrically. Sigmodal acid, in agreement with the results of Dille and Koppanyi (5), gives a lighter color in the cobalt test than barbital, this being due to the difference in the molecular weights. Since the cobalt color test is not specific for any particular barbiturate, there is no proof that sigmodal is excreted in the urine unchanged. If it is not changed in the

body, the relatively high percentage excretions recorded above are correct, but if it is converted to a barbiturate of lower molecular weight, the results would be correspondingly lower. Even these lower figures, however, would be sufficient to warrant a statement that sigmodal is excreted in the urine in appreciable quantities.

The margin of safety of sigmodal sodium as seen from the tables is comparable to that of other barbiturates. The margin of safety represents the ratio between the fatal and the 4+ anesthetic doses. This margin is wide enough to allow the free use of sigmodal sodium as a laboratory anesthetic. Dogs may be anesthetized safely with doses from 25 to 30 mg. by vein, and a good surgical anesthesia may be maintained for two hours or more. The operated animals show an uneventful recovery in about five hours. While sigmodal sodium may be unreservedly recommended as a laboratory anesthetic, it should not be used to produce surgical anesthesia in humans. No fixed anesthetic should be used for this purpose. Let us state definitely not only with reference to sigmodal sodium but to other barbiturates, that since small differences in potency of barbiturates cannot be detected with certainty and since animals show considerable variations in effects produced by the same dose of a barbiturate, it is impossible to calculate a safe average anesthetic dose for humans, particularly in view of the relatively narrow margin of safety of barbiturates. These considerations apply only to the use of barbiturates for complete surgical anesthesia and not to their use in pre-anesthetic medication.

Sigmodal sodium is definitely a short-acting barbiturate. As is the case with most barbiturates, it is shorter-acting in rabbits than in dogs. To study the duration of action, *i. e.*, the essential elimination of sigmodal, the method of Koppanyi and Lieberson (7) was used and also a new method which is believed to be applicable to all central depressants. This method is very simple and presupposes only the knowledge of the 50 per cent fatal dose, the average sleeping time of the survivors from this dose, and of the maximum dose which permits the maintenance of normal body posture. Since by the term "recovery" we mean the ability of the animal to maintain its normal posture unassisted at the termination of sleep, we assume that the animal is in the same condition as an animal which has received the maximum amount of the drug not interfering with its ability to maintain normal body posture. Therefore, the formula —50 per cent fatal dose minus maximum dose permitting normal body posture, divided by the sleeping time of the survivors—discloses the amount of drug essentially eliminated per unit of time. The results obtained by the two different methods agree fairly closely (18 mg. *vs.* 15 mg. per hour).

Determination of the essential elimination of a hypnotic by the intravenous method should enable one to predict the average sleeping time by the oral, rectal or other routes of administration when anesthetic doses are given, provided there is complete absorption of the drug. The results from the second method show that 15 mg. of sigmodal sodium are essentially eliminated during one hour. By modifying the formula to calculate for sleeping time instead of elimination, it may be estimated that rabbits receiving 90 mg. of sigmodal sodium rectally should recover in 320 minutes $\left(\frac{90 - 10}{15} = 5 \text{ hrs. } 20 \text{ min.}\right)$; rabbits receiving 80 mg. should recover in 280 minutes, and rabbits receiving 60 mg. should recover in 200 minutes. Considering the relatively small number of animals used for the determination of sleeping time in Table II, the agreement between the theoretical and the actual sleeping times is remarkably close. According to Boedecker and Ludwig (8), rabbits receiving 400 mg. of sigmodal orally recover from their narcotic sleep in 24 hours or more. By the above method of calculation $\left(\frac{400 - 10}{15} = 26 \text{ hours}\right)$, the theoretical time of recovery again closely approximates the actual experimental results. From protocol data available in the laboratory on 3,3-diethyl-2,4-dioxotetrahydropyridine predictions were made on the sleeping time from various doses; these checked well with the experimental observations. We feel, there-

fore, that this method of estimating sleeping times may be applied to other central depressants.

The narcosis produced by sigmodal is similar to that produced by other short-acting barbiturates. It should be emphasized, however, that in rabbits the onset of action by rectal administration is very rapid and fairly constant. This agrees with the results of Boedecker and Ludwig (8) and Reichold (9).

The effects of this drug on the heart, circulation and respiration are not different from those of other barbiturates. We mentioned the fact that fatal doses may produce a rather unique type of Cheyne-Stokes respiration where the periods of respiratory movements and of the apnœa are very prolonged.

Sigmodal sodium suppresses convulsions from antipyrine and strychnine and saves the lives of animals from four fatal doses of strychnine given by a single injection. This indicates a rather powerful anti-convulsant action. Antipyrine, which is added by the manufacturer to the standard rectal solution of sigmodal sodium, does not appear to increase its toxicity or its analgesic effect.

Metrazol was found to be an efficient analeptic in sigmodal sodium narcosis. It could be and already has been (10), (11), (12), (13) used with benefit in sigmodal poisoning and in conditions in which the immediate interruption of sleep appeared to be desirable.

SUMMARY

1. The 50 per cent fatal dose of sigmodal sodium for rabbits is 40 mg. (intravenous) and 90 mg. (rectal), and for dogs, about 35 mg. (intravenous).

2. Sigmodal sodium is a depressant of the central nervous system producing in appropriate doses in rabbits loss of righting reflexes, motor paralysis, muscular relaxation and a deep sleep from which the animals cannot be aroused. In the dog it produces true surgical anesthesia.

3. The onset of action of sigmodal sodium after intravenous injection is immediate in all animals and after rectal administration it occurs within a few minutes in rabbits.

4. The rate of essential elimination of sigmodal sodium was found to be 37 to 47 per cent of the average fatal dose (15 to 19 mg.) per hour as determined by two different methods, one of which is described herein for the first time.

A method for estimating sleeping time after rectal, oral and other routes of administration applying the data derived from the intravenous essential elimination determination is described.

5. The drug given intravenously is a circulatory and respiratory depressant comparable to other short-acting barbiturates. The blood pressure and respiratory rate in animals anesthetized with sigmodal sodium are similar to those obtained under other anesthetic agents.

6. Sigmodal sodium, even in fatal doses, does not abolish the cardiac slowing from faradic stimulation of the vagus. The cardiac and vasomotor responses to autonomic drugs are unchanged.

7. Sigmodal sodium has definite anti-convulsant properties and conversely, a central stimulant such as metrazol antagonizes its depressant effects.

8. Large doses of antipyrine do not deepen sigmodal sodium narcosis or alter the recovery time.

9. Sigmodal sodium may be estimated in body fluids by the cobalt-color tests. About one-fifth of the dose of the drug (or its end products) is excreted in the urine within 48 hours following administration.

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Further Observations on the Influence of the Anesthetic on the Results of Digitalis Assay by the Cat Method of Hatcher and Brody

By Chas. C. Haskell

In 1936, data were presented, which established that the cat unit for certain preparations of digitalis was materially higher when a non-volatile anesthetic, dial-urethane solution, was substituted for ether in the Hatcher-Brody method (9). The only earlier published reports regarding the possible influence of a non-volatile anesthetic on the size of the cat unit encountered were in the papers of Epstein (4), David and Rajaminickam (2), and Bauer and Fromherz (1), but, with the possible exception of the last-mentioned, the number of experiments performed by these authors seems scarcely to justify the conclusions drawn. This criticism is not applicable to the later publication by Edmunds, Moyer and Shaw (3), in which it was pointed out that, with animals under urethane anesthesia, the cat unit for the International Powder was considerably higher than was the case when etherized animals were used. The practical importance of departing from the original technique of the Hatcher-Brody method by substitution of a non-volatile anesthetic is immediately obvious; in addition, the interesting question arises why etherized cats succumb to smaller doses of digitalis than do those under the influence of dial-urethane or urethane. Does ether in some way directly lower the resistance of the cats to digitalis poisoning or, on the other hand, do the non-volatile anesthetics mentioned oppose, in some specific manner, the lethal action of this drug? Another possibility, suggested by Dr. Harry Gold (5), is that the influence of the non-volatile anesthetic is indirect; by prevention of struggling, it delays the onset of ventricular fibrillation, so prone to be precipitated by a struggle in unanesthetized cats after large doses of digitalis. If it is found that other non-volatile anesthetics, chemically unrelated to dial or urethane, affect the resistance of cats to digitalis as do these latter, it would tend to support, but not establish the correctness of Dr. Gold's explanation.

David and Rajaminickam (2) assayed a specimen of the International Powder on five series of cats, using ether, urethane, chlorobutanol, chloralose or paraldehyde in the different series. They report that the chlorbutanol series gave the largest cat unit; the smallest being obtained from the chloralose series; while that from the etherized cats was intermediate in size. Such results would seem to indicate that the various anesthetic agents acted in a specific manner to affect the resistance of the cats to digitalis intoxication, but when it is taken into consideration that the number of animals in each series was only five, except in case of paraldehyde, where four were used, it is obvious to anyone familiar with the practical use of this method that the differences recorded are without significance. It is of interest to note that David and Rajaminickam found the ether cat unit larger than when urethane was used, in contrast to the results obtained by Edmunds, Moyer and Shaw (3) from a much larger number of animals. As already stated (8) a limited number of experiments in our laboratory in which the cat unit for animals anesthetized with chlorbutanol was compared with that obtained with etherized cats, failed to show any material difference, but here, too, the number of experiments was far too small to justify positive conclusions. It seemed desirable, therefore, to make further observations, in the attempt to determine whether the substitution of chlorobutanol for ether anesthesia affects the size of the cat unit.

EXPERIMENTAL

In the first experiments, the procedure was similar to that employed in comparing results under dial-urethane and ether anesthesia. Groups of ten cats were used for each assay; five of the animals received 200 mg. chlorbutanol per Kg. intraperitoneally, a 40% solution in ethylene glycol being used; the other five being lightly etherized. The

digitalis solution, diluted with 0.9% sodium chloride, was injected into the femoral vein at the rate of approximately 1 cc. per 2.5 Kg. body weight every 2½ minutes. The results of these experiments are given in Table I.

Examination of these tabulated results shows that the cat unit for five of the six preparations tested was larger when chlorbutanol anesthesia was used. In the case of Glu. 1036–5313, the difference is small and with Tr. Digitalis S.26, the "ether unit" was actually larger than the "chlorbutanol unit." It should be borne in mind, however, that the cat units given here represent the average from groups of only five animals; as will be shown later, assays of Tr. Digitalis S.26 on forty cats under chlorbutanol anesthesia gave a cat unit of 12.53 cc. of the diluted solution. Even if the supposedly "aberrant" results with Tr. Digitalis S.26 are included and the differences expressed as percentages of the smaller cat unit, it is found that the "chlorbutanol unit" is 24.6% larger than the "ether unit." A similar comparison between the results with dial-urethane and ether anesthesia, seven different digitalis preparations being used on a total of ninety-nine cats, showed that the "dial-urethane unit" was consistently larger, the average percentage difference amounting to 30.4 (8).

Because of the greater convenience, dial-urethane solution was substituted for ether in a continuation of the experimentation, 0.6 cc. of this solution per Kg. being given to five of each group of ten cats; the others receiving chlorbutanol. Three preparations of digitalis were used; one assay was carried out on two of each of these and eight were carried out on the third; Tr. Digitalis S.26. The results are given in Table II.

With the exception of the third assay of Tr. Digitalis, S.26, the differences in the size of the cat units with the animals under chlorbutanol or dial-urethane anesthesia are slight and lie well within the limit of error for the method when only five cats are used in each assay. In view of the fact that the remaining thirty-five cats anesthetized with chlorbutanol and used for the assay of Tr. Digitalis, S.26 gave a cat unit of 12.81 cc., it is probable that the figure obtained from the average of the individuals in the third group, 10.50 cc., is too low. The separate results in this group were 8.95, 9.67, 11.07, 11.30 and 11.49 cc., so that the use of Van Wijngaarden's formula, in which such implicit confidence seems placed by certain authors, fails to support the view that the cat unit does not represent the true strength of the tincture (12).

Taking the average of all the group assays, the difference between that for the chlorbutanol groups, 12.69 cc., and the dial-urethane groups, 13.06 cc., is only 0.37 cc., or slightly less than 2.9% of the smaller average. From these results, as well as from the direct comparison between chlorbutanol and ether, it seems safe to conclude that the cat unit for a digitalis preparation, as determined on animals under the influence of chlorbutanol anes-

Table I.—Results of Assays under Chlorbutanol and Ether Anesthesia

Preparation	Cat Unit[a]		Percentage Difference
	Chlorbutanol	Ether	
Glucosides 1036–5313	9.88 cc.	9.56 cc.	3.3
Ampule 5 cc. Sp.	15.25 cc.	10.67 cc.	42.9
Ampule K.	11.97 cc.	8.76 cc.	36.6
Tr. Digitalis W., Conc.	6.15 cc.	3.99 cc.	54.1
Tr. Digitalis, S.26	9.14 cc.	10.16 cc.	−11.2
Tr. Digitalis, S. K.36	10.82 cc.	8.87 cc.	21.9
	Average percentage difference[b]		24.6

[a] The Cat Unit is expressed in cc. of the diluted solutions used.
[b] The "average percentage difference" is obtained by taking the average of the individual percentage differences.

Table II.—Results of Assays under Chlorbutanol and Dial-Urethane Anesthesia

Preparation	Cat Unit		Percentage[a] Difference
	Chlorbutanol	Dial-Urethane	
Liq. No. 64783	14.72 cc.	15.80 cc.	D- 7.3
Amp. No. 42453	12.01 cc.	12.22 cc.	D- 1.7
Tr. Digitalis, S.26	13.67 cc.	14.05 cc.	D- 2.7
Tr. Digitalis, S.26	11.18 cc.	12.01 cc.	D- 7.4
Tr. Digitalis, S.26	10.50 cc.	13.07 cc.	D-24.5
Tr. Digitalis, S.26	13.70 cc.	12.47 cc.	C- 9.8
Tr. Digitalis, S.26	13.12 cc.	11.84 cc.	C-10.7
Tr. Digitalis, S.26	12.93 cc.	13.33 cc.	D- 3.1
Tr. Digitalis, S.26	13.42 cc.	12.74 cc.	C- 5.3
Tr. Digitalis, S.26	11.70 cc.	13.07 cc.	D-11.7
Average	12.69 cc.	13.06 cc.	...
	Average percentage difference[b]		D- 2.9

[a] The "Percentage Difference" is expressed as percentage the difference is of the smaller cat unit; preceded by "D" indicates that the unit under dial-urethane anesthesia was the larger; preceded by "C," that the chlorbutanol unit was the larger.
[b] The "Average percentage difference" is obtained by determining the percentage that the difference between 12.69, the chlorbutanol average and 13.06, the dial-urethane average, is of the smaller of these two figures; 12.69.

thesia, is definitely higher than that obtained by the use of etherized cats; and further, that no material difference in the size of the cat unit exists whether the determination is made on animals under the influence of chlorbutanol or dial-urethane anesthesia.

The fact that two chemically unrelated non-volatile anesthetics, chlorbutanol and dial-urethane solution produce essentially the same deviation from the cat unit as determined on etherized cats certainly suggests that there is nothing specific in the influence of these drugs in affecting resistance to digitalis poisoning. One must consider the possibility that ether lowers the resistance of the animals, but that this is not the case seems probable from experiments carried out in our laboratory by Haag (7) eleven years ago. Twelve cats, under the influence of nitrous oxide-oxygen anesthesia, were decerebrated by the method of Pollock and Davis (11) and the cat unit for a tincture of digitalis as determined on them was found to be 66.2 mg., as compared with a cat unit of 75 mg. obtained by the use of ten etherized cats. MacDonald and Schlapp (10) later made use of spinal cats, being of the opinion that the animals reacted more uniformly than was the case when ether was employed, but from their paper, it is not clear whether they tested the same tincture on spinal and on etherized cats. Of course, the condition of a decerebrated or spinal animal is far from normal; it is quite possible that the operative procedure may render them less resistant to the toxic action of digitalis; indeed, Gold has shown that hemorrhage has such an influence (6). In our next experiments, therefore, the attempt was made to determine whether deep and rather sustained ether anesthesia prior to and at the beginning of the injection of the digitalis solution, followed by administration of dial-urethane would give a cat unit differing from that obtained with the animals under the influence of this latter throughout.

As in all the preceding experiments, groups of ten cats were used in each assay. Five of each group were allowed to inhale ether until complete cessation of respiration from central action of the drug occurred. In spite of the institution of artificial respiration by means of manual compression, three of the fifty animals succumbed to the ether. The remainder, on their resuming spontaneous respiration, were kept deeply etherized for the operative procedures and until the venous cannulas had been inserted into all members of the group. Five cats of each group received the customary dose of the dial-urethane, 0.6 cc. per Kg. intraperitoneally, with no preliminary etherization. The results of these experiments are given in Table III.

Table III.—Results of Assays under Dial-Urethane Anesthesia Alone or Preceded by Ether

Preparation	Cat Unit: Dial-Urethane Preceded by Ether	Cat Unit: Dial-Urethane Alone	Percentage Difference
Amp. 65483	10.37 cc.[b]	12.55 cc.	21.0
Glu. 1036–3888	10.42 cc.	11.62 cc.	11.5
Glu. 1036–3888	9.13 cc.	10.33 cc.	13.1
Glu. 1036–3888	10.67 cc.	12.38 cc.	16.0
Tr. Digitalis, S.26	11.62 cc.	12.60 cc.	7.6
Tr. Digitalis, S.26	9.85 cc.[b]	12.75 cc.	33.5
Tr. Digitalis, S.26	10.26 cc.	11.31 cc.	10.2
Tr. Digitalis, S.26	12.10 cc.	11.47 cc.	− 5.5[a]
Tr. Digitalis, S.26	11.64 cc.[b]	12.36 cc.	10.6
Tr. Digitalis, S.26	11.73 cc.	11.86 cc.	1.1
		Average percentage difference[c]	10.6

[a] Preceded by minus sign because the cat unit from the animals under dial-urethane is smaller than that from the animals previously etherized.
[b] Average of four cats.
[c] The average percentage difference is obtained by determining the percentage that the difference between 10.78 cc., the average for the series in which ether and dial-urethane were used, and 11.92 cc., the average for the dial-urethane series is of 10.78.

In Table III, it is shown that, with the exception of a single assay of Tr. Digitalis, S.26, the cat unit was smaller when determined on animals that had been etherized prior to administration of dial-urethane solution. The significance of these differences is hard to appraise; omitting the first, fourth and sixth assays, they are so small as to fall within the limit of error for the method when only five animals are used. The largest differences were encountered in the first and sixth assays; in both these, only four cats were available for the ether-dial-urethane groups, the fifth animal in each group having succumbed to ether poisoning. Another possible contributing factor in bringing about the differences was the method of manual compression employed after respiration had failed in the etherized cats; unfortunately, the condition of the lungs was not examined post morten. Disregarding these considerations, however, it is found that the average from all of the cats anesthetized with dial-urethane alone is not very much larger than that from the animals that had been etherized before injection of the dial-urethane solution; the former being 11.92 cc. and the latter, 10.78 cc., the difference being 1.14 cc. or 10.6% of the smaller average. It seems safe to conclude, therefore, that if ether does lower resistance of cats to poisoning by digitalis, under the conditions of the above described experiments, it does not do this to any material extent.

SUMMARY

Briefly summarizing, it would appear that: The cat unit for the digitalis preparations examined is, on the average, considerably higher if chlorobutanol anesthesia is substituted for ether in the performance of the assay by the cat method of Hatcher and Brody.

The cat unit, as determined on cats under the influence of chlorbutanol anesthesia, is practically identical with that obtained by the use of animals under dial-urethane anesthesia.

Profound etherization of the animals prior to administration of dial-urethane for anesthesia does not lead to a reduction of the size of the cat unit to any marked extent if a non-volatile anesthetic, dial-urethane, is subsequently given in dose sufficient to prevent struggling.

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The U. S. P. XI Digitalis Standard

*By Orlo F. Swoap and Marvin L. Pabst**

The adoption in the Eleventh Decennial Revision of the United States Pharmacopœia which became official June 1, 1936, of a standard reference digitalis powder to replace ouabain as a standard for digitalis preparations naturally led to the question of whether any pronounced changes in the activity of official preparations had occurred in the transition from the U. S. P. X.

The growing confusion among physicians and others with regard to the strength of official U. S. P. XI digitalis preparations in terms of the familiar U. S. P. X standard has been recently pointed out by Wright, Fahr, and Lewis (1) in their correspondence to the editor of the *Journal of the American Medical Association*. It therefore, appears timely for us to call attention to conclusions reached in our laboratory on this matter.

We have used the U. S. P. XI Reference Digitalis Powder (No. 3057 × 915921) in this laboratory since it was obtainable. This powder carries the correction factor or relationship that 0.0745 Gm. is equivalent to 1 "U. S. P. Digitalis Unit" or 0.1 Gm. of the International Standard Digitalis Powder. We wish to call particular attention to the fact that this correction factor has always been applied in preparing extracts from the powder so that in all cases we have used a strictly U. S. P. XI Standard Tincture.

Our first tests, when we received this powder in April, 1936, indicated the U. S. P. XI Standard Tincture to be approximately 125 per cent U. S. P. X. Other laboratories (2), (3), (4) had reported that the International Standard was about 125% of the U. S. P. X standard and it was therefore considered that the digitalis standard had been raised 20–30 per cent over the U. S. P. X level. Later parallel runs between the U. S. P. XI standard tincture digitalis and ouabain indicated this value to be somewhat higher. Hence, it was decided to directly compare the U. S. P. XI standard tincture to ouabain at various times and thus by a series of comparative tests, spread over a sufficient time interval, to accurately determine the relationship between these two digitalis standards.

EXPERIMENTAL

Our method of testing these preparations has been that specified in the U. S. P. XI, pages 397–398.

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We store frogs in a refrigerator at a temperature of 5–10° C., in trays supplied with running water, and then place them in a constant-temperature tank as specified in the U. S. P. XI (temperature 20° C. ± 1.0° C.). Our refrigerator and constant-temperature equipment is that supplied by the George H. Wahmann Manufacturing Company of Baltimore, Maryland, and corresponds to that in use at the Food and Drug Administration and several other laboratories. We have found, however, that due to the tremendous variation in seasonal susceptibility of the frog (*R. pipiens*), it has been impossible to hold to the specifications of the U. S. P. XI, page 398, which state that the injected dose of diluted digitalis standard tincture shall be equivalent to not less than 0.003 cc. and to not more than 0.008 cc. of the original standard preparation per Gm. of body weight of frog (see Table I). Runs are made on groups of ten frogs for each single dose and the 50 per cent Minimum Systolic Dosages are determined from the observed mortalities by the use of Trevan's Curve (5), which has been shown by others (6), (7) to hold for digitalis preparations and ouabain. This 50 per cent M. S. D. value is then used for comparison of standard and unknown and the determination of percentage values. All runs reported in this paper have been made in parallel on both the standard tincture and ouabain.

Our work has now covered a period of two years, and during its progress other laboratories reported figures to indicate that the digitalis standard had been raised approximately 50–60 per cent in changing from U. S. P. X to U. S. P. XI (8), (9), (10). The average of our values as shown in Table I indicates that U. S. P. XI digitalis preparations are 160 per cent U. S. P. X.

Table I.—Comparison of U. S. P. XI Standard Tincture Digitalis to Ouabain Using the U. S. P. One-Hour Frog Method

	U. S. P. XI Standard Tincture Digitalis			Ouabain			
Date	Dose Cc./ Gm.	Mortality Per Cent	50 Per Cent M. S. D.[a] Cc.	Dose Mg./ Gm.	Mortality Per Cent	50 Per Cent M. S. D.[a] Mg.	Per Cent XI of X
3–15–37	0.0045	70	0.00414	0.00060	40	0.000637	185
3–15–37	0.0040	60	0.00384	0.00065	40	0.000690	216
3–22–37	0.0040	45	0.00412	0.00070	50	0.000700	204
3–22–37	0.0040	50	0.00400	0.00070	50	0.000700	210
3–24–37	0.0040	40	0.00424	0.00070	60	0.000672	190
4–28–37	0.0040	50	0.00400	0.00070	30	0.000790	237
8–10–37	0.0090	50	0.00900	0.00080	30	0.000903	120
8–10–37	0.0090	40	0.00954	0.00090	40	0.000954	120
9–24–37	0.0085	30	0.00959	0.00075	40	0.000795	100
9–24–37	0.0095	40	0.01008	0.00080	60	0.000767	91
9–28–37	0.0095	50	0.00950	0.00075	20	0.000929	117
9–28–37	0.0095	40	0.01008	0.00090	40	0.000954	114
11– 9–37	0.0075	60	0.00719	0.00080	40	0.000848	141
11– 9–37	0.0070	50	0.00700	0.00085	50	0.000850	146
12–17–37	0.0050	40	0.00531	0.00070	50	0.000700	158
12–23–37	0.0050	50	0.00500	0.00070	50	0.000700	168
12–23–37	0.0050	30	0.00564	0.00070	40	0.000743	158
12–28–37	0.0050	50	0.00500	0.00070	40	0.000743	178
12–28–37	0.0050	40	0.00531	0.00075	40	0.000795	180
1–13–38	0.0050	40	0.00531	0.00070	50	0.000700	158
1–13–38	0.0055	40	0.00583	0.00070	40	0.000743	153
1–26–38	0.0050	40	0.00531	0.00070	30	0.000790	179
1–26–38	0.0055	40	0.00583	0.00080	50	0.000800	165
2– 8–38	0.0050	50	0.00500	0.00080	40	0.000848	203
2– 8–38	0.0050	30	0.00564	0.00085	50	0.000850	181
2–10–38	0.0055	50	0.00550	0.00085	40	0.000902	197
2–10–38	0.0055	40	0.00583	0.00090	80	0.000795	164
2–25–38	0.0055	50	0.00550	0.00085	50	0.000850	185
2–26–38	0.0055	80	0.00486	0.00085	40	0.000902	222
3– 3–38	0.0050	80	0.00442	0.00085	40	0.000902	245
3–25–38	0.0060	60	0.00576	0.00095	60	0.000911	190
4– 1–38	0.0055	50	0.00550	0.00090	50	0.000900	196
4– 1–38	0.0055	50	0.00550	0.00090	40	0.000954	208
4–27–38	0.0065	70	0.00598	0.00095	70	0.000875	176
4–27–38	0.0060	50	0.00600	0.00090	30	0.001017	203
5– 3–38	0.0060	60	0.00576	0.00095	60	0.000911	190
5– 3–38	0.0060	60	0.00576	0.00095	50	0.000950	198

Table I.—(*Continued*)

5-13-38	0.0060	30	0.00667	0.00095	50	0.000950	168
5-13-38	0.0070	40	0.00743	0.00095	80	0.000839	136
5-25-38	0.0090	70	0.00828	0.00100	80	0.000884	128
6-16-38	0.0080	50	0.00800	0.00095	70	0.000875	131
6-16-38	0.0080	40	0.00848	0.00090	50	0.000900	127
7- 7-38	0.0085	60	0.00815	0.00090	80	0.000795	117
7- 7-38	0.0085	60	0.00815	0.00080	40	0.000848	125
7-12-38	0.0085	70	0.00782	0.00085	50	0.000850	130
8-16-38	0.0095	35	0.01039	0.00090	40	0.000954	110
8-16-38	0.0100	60	0.00959	0.00095	30	0.001073	134
9- 8-38	0.0085	50	0.00850	0.00090	70	0.000828	117
9- 8-38	0.0085	60	0.00815	0.00080	50	0.000800	118
9-20-38	0.0080	40	0.00848	0.00080	70	0.000737	104
9-20-38	0.0085	40	0.00902	0.00075	40	0.000795	106
11-17-38	0.0070	80	0.00618	0.00075	50	0.000750	146
11-17-38	0.0060	50	0.00600	0.00075	60	0.000719	144
11-29-38	0.0050	30	0.00564	0.00070	60	0.000672	143
12-21-38	0.0045	40	0.00477	0.00065	60	0.000623	156
12-21-38	0.0050	50	0.00500	0.00065	50	0.000650	156
1-11-39	0.0045	30	0.00508	0.00065	30	0.000734	173
1-31-39	0.0045	30	0.00508	0.00070	70	0.000644	152
1-31-39	0.0050	70	0.00460	0.00065	50	0.000650	170
2-15-39	0.0045	60	0.00432	0.00065	60	0.000623	173
						Average	160

[a] As calculated from Trevan's Curve (5).

An examination of this table shows a tremendous divergence of results from the low value of 91 per cent X to the high value of 245 per cent X. These variations far exceed experimental error and appear to have a bearing on the time of year that the comparisons were made. By plotting the daily average percentage values U. S. P. X for the standard tincture, it is easily seen that this appears to be in the nature of a seasonal variation. Further work on this matter is being continued.

Our work thus shows that in order to arrive at any definite comparison of the U. S. P. X and XI standards for digitalis, observations must be made over a relatively long period of time. Our indication that the U. S. P. XI standard tincture digitalis is 160 per cent U. S. P. X is in excellent agreement with that of Rowe and Pfeifle (10), who have reported a value of 151 per cent U. S. P. X covering a one-year period. It is interesting to point out that our work was started at a point where Rowe and Pfeifle left off and had been in progress a year before the details of their work were published. If their values in Table I of their paper (10), page 183, were plotted against time, it would appear that our curve is a direct continuation of their curve.

The work of both laboratories, covering a three-year period, would therefore indicate that, in changing from U. S. P. X to U. S. P. XI, the potency of official digitalis preparations has been increased 50 to 60 per cent.

During the course of the experiments here described, the U. S. P. XI Standard Reference Digitalis Powder No. 3057 × 915921, the Canadian Standard Digitalis Powder No. 1135, and the International Standard Digitalis Powder (1936) were compared using their appropriate factors.

Table II.—Comparison of Official Digitalis Standards

Date	Preparation	Per Cent International Tincture	
9-28-37	International Standard Tincture Digitalis		100
	U. S. P. XI Standard Tincture Digitalis	106.1 105.3	av. 105.7
	Canadian Standard Tincture Digitalis	100.0 106.1	av. 103.1

The results given in Table II, based on two comparisons made on the same day, indicate that the present U. S. P. and Canadian Digitalis Standards are approximately equal to the International Standard.

Ouabain was also included in the above test permitting the comparison in terms of U. S. P. X values. At the date of this assay, the International Standard Powder (1936) was found to be 109 per cent U. S. P. X, the U. S. P. XI Standard Powder 116 per cent U. S. P. X, and the Canadian Standard Powder (No. 1135) 113 per cent U. S. P. X. These values again show the equality of the three Digitalis Standard Powders. The low U. S. P. X values have been explained in the first part of this paper (see Table I).

The limited results as shown in Table II are in agreement with the U. S. P. XI statement that the present U. S. P. and International Digitalis Standards are identical in potency. This would indicate that the International Standard, at the time of its adoption, was set at a much higher level than previously accepted clinical standards.

SUMMARY

The U. S. P. XI Digitalis Standard has been compared to the U. S. P. X Digitalis Standard in a series of parallel runs extending over a period of two years.

The results show that the U. S. P. XI Digitalis Standard averages about 50 to 60 per cent stronger than the U. S. P. X Digitalis Standard.

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A Recent Substitute for Jalap*

By Heber W. Youngken

A little more than a year ago the writer received a sample of a root from a pharmaceutical manufacturer who claimed to have purchased it as "Jalap," but which after having been made into a preparation yielded a product so unlike that which the manufacturer was accustomed to recognizing as the Jalap preparation that his suspicions were aroused. Never before had Jalap been found to yield so low a percentage of resin.

The sample forwarded for identification consisted of transverse segments of a root, the cut surfaces of which possessed a dirty white to pale brown color and exhibited concentric rings of bundles. The segments were lighter in weight than Jalap cut similarly. A cursory examination with a hand lens failed to disclose any evident resin cells.

Cross and longitudinal sections were prepared from it and examined in water, chloral and in phloroglucin-HCl mounts. These showed it to possess a starch- and crystal-bearing parenchyma imbedded in which were a concentric series of open collateral bundles. Typical resin cells, closely arranged, tracheæ with bordered pores, rosette aggregates of calcium oxalate and characteristic starch grains such as Jalap possesses were absent. It differed from Poke, Beet, Amaranthus, Levant and Mexican Scammony, Turpeth Root, Piptostegia and other roots exhibiting concentric circles of bundles with which the writer was familiar. The only tangible clues from a microscopic examination of its structure were the presence of numerous raphides of calcium oxalate, many of which occurred in bundles within crystal cells of the parenchyma, and its possession of a concentric series of secondary collateral bundles.

A search of the literature of pharmacognosy failed to reveal anything recorded which corresponded with this sample.

A possible clue remained. If this material was marketed as "Jalap," it may have been shipped from Jalap-producing Mexico. In thinking of some of the plants commonly found growing there, *Mirabilis Jalapa* flashed to mind. Upon looking up the literature on the anatomy of the axis of this plant, I came upon a brief reference in Solereder's Systematic Anatomy of the Dicotyledons (1). Raphides of calcium oxalate and the concentric series of bundles were there mentioned as occurring in *Mirabilis* species. I obtained some roots of *M. Jalapa* from the Medicinal Plant Garden of the University of Minnesota through the kindness of Professor Fischer. Upon sectioning one of these and comparing its histology with that of the

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unknown sample, the identity was revealed. It proved to be the root of *Mirabilis Jalapa* (Fam. *Nyctaginaceæ*).

The characteristics of this root and of the plant yielding it will now be described.

ROOT OF MIRABILIS JALAPA

Physical Characteristics.—In their entire condition, the roots occur either singly or in clusters of two or more. Single roots, tuberous, conical, a number of them resembling roots of Aconites in form, up to 20.3 cm. in length and up to 5 cm. in width at the crown, the crown somewhat rounded and bearing one or more stem bases, the lower portion tapering into a conical tap root. Clustered roots show branching in upper part of primary root, forming in the lot examined from 2 to 3 secondary roots which remain attached to the parent tuberous root and are themselves tuberous. The external surface of these roots is dark brown to blackish brown, transversely and longitudinally wrinkled and showing few to numerous lenticels. The fracture is hard, complete and horny, the fractured surface exhibiting a prominent dark brown cork and a broad whitish cortical and wood region, the latter marked by concentric rings of vascular tissue.

Histology (see Fig. 2).—Transverse sections exhibit a cork region of up to about 20 layers of brick-shaped cork cells having suberized walls and brownish contents, a phellogen of meristematic cells, a cortex of up to many layers of parenchyma, most of the cells of which contain starch, a large number of raphides of calcium oxalate appearing silky in unstained mounts and a scattering of cells with resinous contents. In older portions of these roots secondary phellogens were found at varying levels in the cortex which had layed down cork on their outer faces, forming borke. Beneath the cortex occurs a broad zone of pericyclic parenchyma in which a concentric series of secondary cambia have layed down vascular tissue forming a number of interrupted circles of secondary open collateral bundles. The conjunctive tissue (pericyclic parenchyma) is composed of cells and intercellular air-spaces, the cells having simple pits and containing either starch, resinous material or raphides of calcium oxalate.

The phloem portion of the bundles consists of sieve tubes, companion cells and phloem parenchyma. The xylem portion consists of radially placed xylem strands composed of xylem cells, and groups of reticulate tracheæ, the latter often radially arranged. Isolated reticulate tracheæ were also frequent in the xylem strands. The largest trachea observed measured 157μ in diameter.

Radial longitudinal sections showed the tracheæ to be largely of the reticulate type. In these sections, a large number of raphide sacs of oblong-elliptic outline were to be noted. The raphide bundles were up to 187μ in length. While a few simple pitted tracheæ occur, they were not seen in the majority of the sections. The cork cells in tangential sections were polygonal in outline. No lignified fibers or stone cells were observed.

Powdered Mirabilis Jalapa Root (see Fig. 3).—Gray to grayish brown. The predominating histological elements were numerous raphides of calcium oxalate, isolated and in bundles, the individual crystals being up to about 160μ in length, starch grains and starch- and crystal-bearing parenchyma. The starch grains were found to be of three types, *viz.*, simple, 2- to 8-compound and oval to spheroidal aggregates. The individual grains varied from

Fig. 1.—Root of the *Mirabilis Jalapa* Linné. *a*, one of several concentric rings of bundles; *b*, lateral secondary root; *c*, lenticel; *d*, longitudinal ridge.

polygonal to plano-convex to angular-convex and were mostly up to 12μ in diameter. Each possessed a central cleft hilum. Other elements observed were brownish fragments of cork, the cells being polygonal to rectangular in outline and with suberized walls; occasional small, brown, amorphous masses of resin, fragments of reticulate tracheæ and nonlignified fibers. The odor was peculiar and unlike anything else with which the writer is familiar. The taste was slightly acrid, followed by a tingling sensation and warmth and stimulating the flow of saliva. When moistened and applied to the skin the powder produced a burning sensation. Within ten minutes after smelling and tasting the powdered material the writer experienced a burning sensation in the nostrils, mouth and fauces. This substitute in powdered form is an irritant to the mucosa and skin.

Description of Plant.—Mirabilis Jalapa Linné, commonly known as Four o'clock or Marvel of Peru, is a perennial herb of bushy form, native to Peru and other parts of Tropical America. It occurs

in Mexico and is commonly cultivated as an ornamental plant in this country. It was named and described briefly by Linnaeus (2) who unfortunately called its specific epithet, "*Jalapa*" because its tuberous roots were once supposed to be the source of the drug Jalap (3). The plant attains a height of 2 to 3 ft., its stems bearing opposite, ovate, to ovate-lanceolate, short-petioled leaves and long, narrow-tubed, funnel-shaped flowers which vary from purple, to variegated purple, red, yellow to white. The showy colored part of the flower represents the calyx. The flowers open in cloudy weather or late in the afternoon, whence the vernacular name of "Four o'clock." The species differs from others in the genus *Mirabilis* by producing only one flower in each involucre.

Assay.—The powdered drug was assayed according to the method for the assay of Ipomœa in the National Formulary (4) and found to yield 2.78% of total resins.

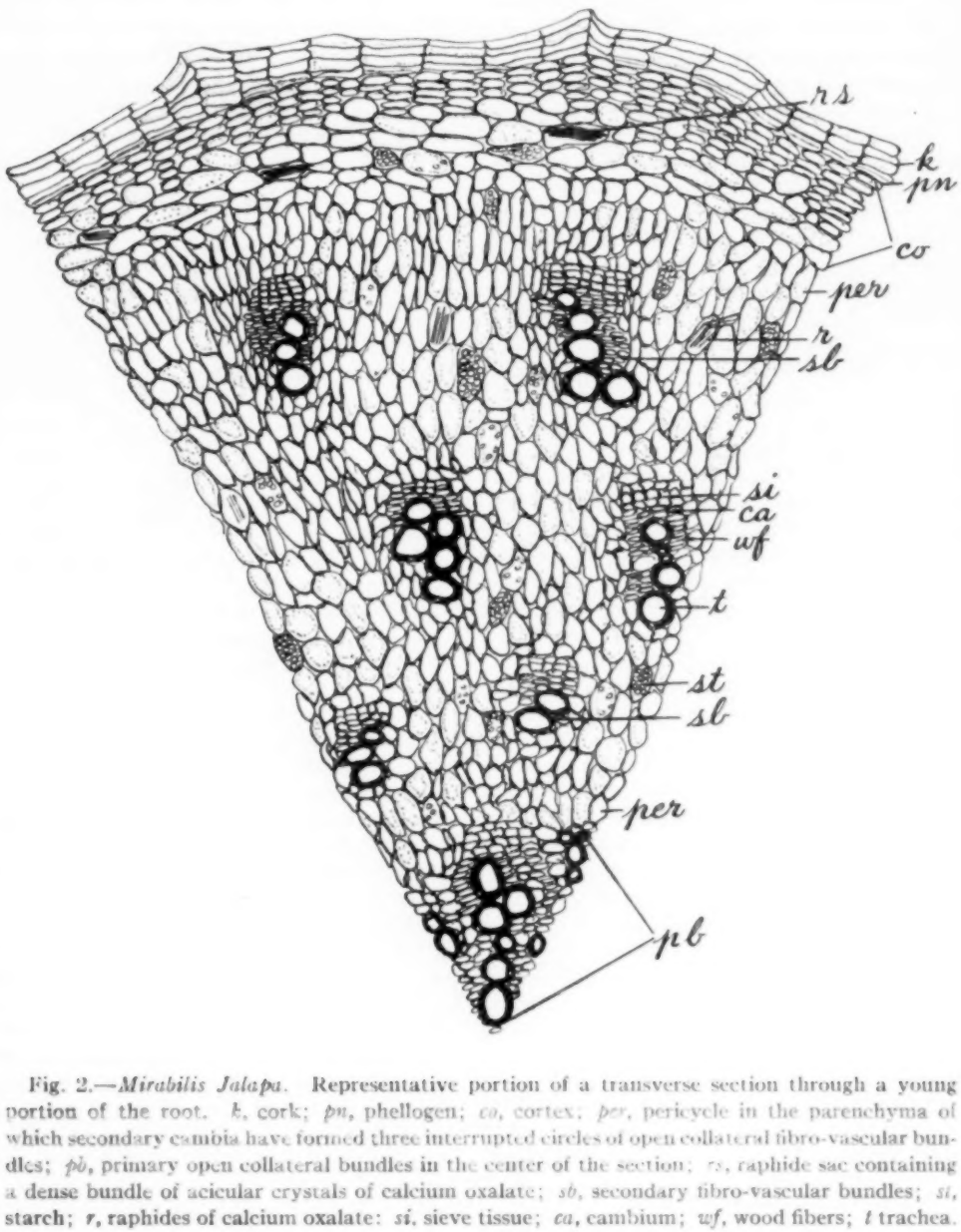

Fig. 2.—*Mirabilis Jalapa*. Representative portion of a transverse section through a young portion of the root. *k*, cork; *pn*, phellogen; *co*, cortex; *per*, pericycle in the parenchyma of which secondary cambia have formed three interrupted circles of open collateral fibro-vascular bundles; *pb*, primary open collateral bundles in the center of the section; *rs*, raphide sac containing a dense bundle of acicular crystals of calcium oxalate; *sb*, secondary fibro-vascular bundles; *si*, starch; *r*, raphides of calcium oxalate: *si*, sieve tissue; *ca*, cambium; *wf*, wood fibers; *t* trachea.

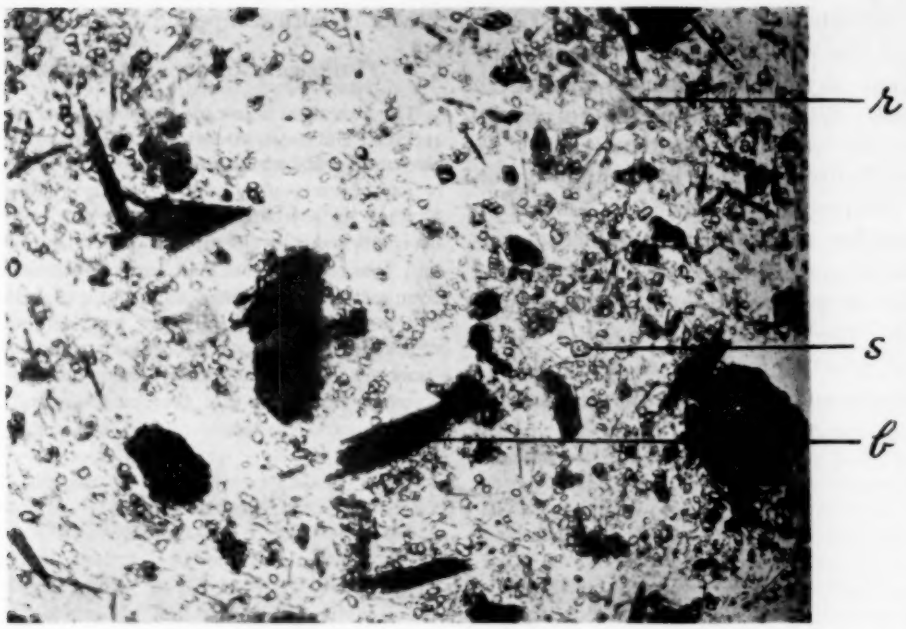

Fig. 3.—Powdered *Mirabilis Jalapa* Root. Photomicrograph X 100. *r*, raphides of calcium oxalate; *s*, starch; *b*, bundle of acicular crystals of calcium oxalate.

SUMMARY

1. A fraudulent substitute for Jalap has appeared on the American market which is identified as the tuberous root of *Mirabilis Jalapa* L.

2. The physical characteristics, histology, and the powdered root of *Mirabilis Jalapa* are described.

3. It is found that the non-lignified character of the cork, the presence of numerous raphides of calcium oxalate, the relatively small amount of resin, the presence of characteristic starch grains, some of which appear in spherical and oval aggregates, and the absence of rosette aggregates of calcium oxalate, bordered pored tracheæ and absence of lignified fibers and stone cells readily distinguish it from Jalap.

4. An assay showed this substitute to contain 2.78% of total resins.

5. It has been determined that the powdered root of *Mirabilis Jalapa* is an irritant to the skin and mucous membrane.

6. A description of the plant yielding the substitute is given.

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A Study of *p*-Nitrosothymol and *p*-Aminothymol*,†

*By W. Taylor Sumerford‡ and Walter H. Hartung***

Thymol because of its availability, therapeutic activity and lack of toxicity has re-

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† Constructed from a portion of a thesis presented by W. Taylor Sumerford to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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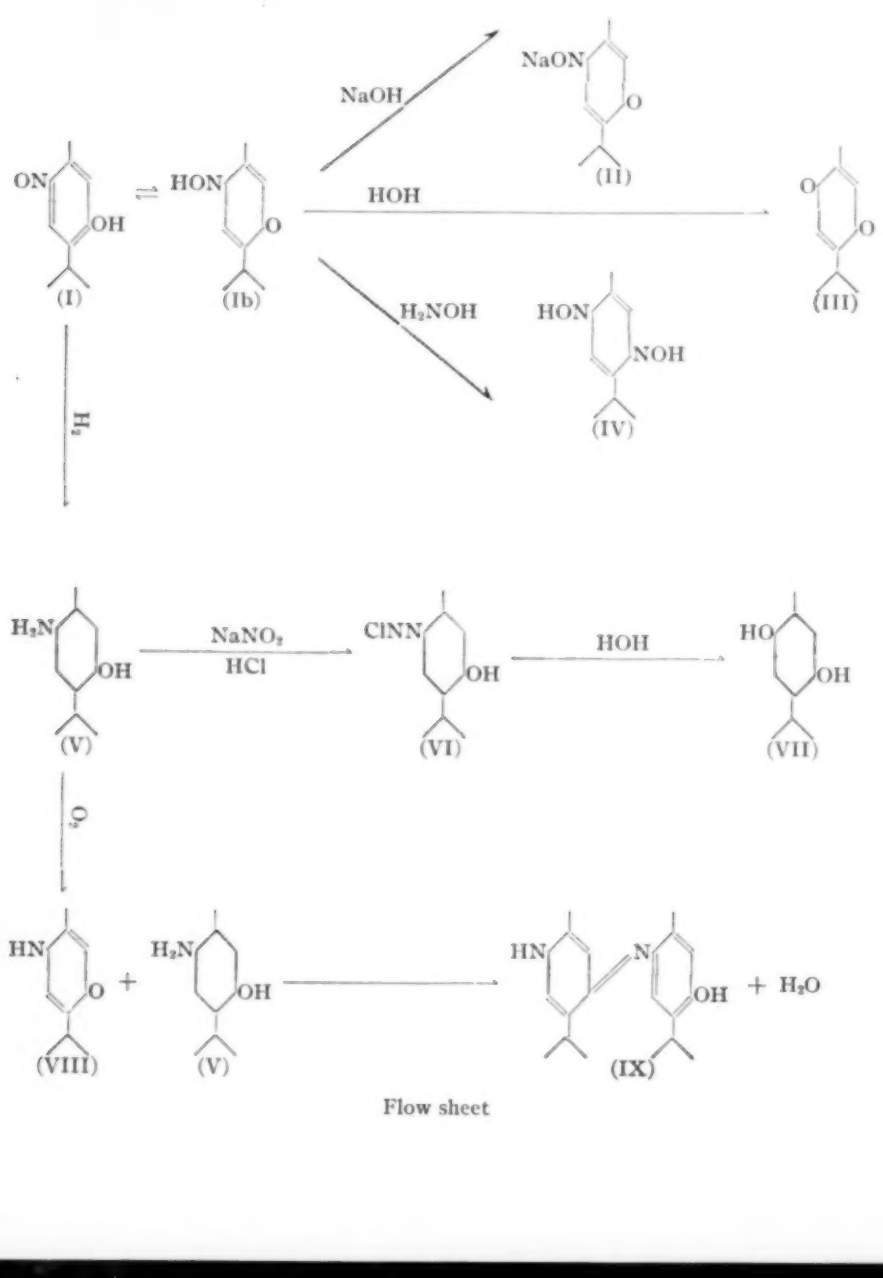
ceived a good deal of attention from pharmacists, chemists and pharmacologists. As a result of this work a number of medicinally important thymol derivatives have been prepared. While nitrosating thymol and reducing the resulting *p*-nitrosothymol by means of hydrogen sulfide to *p*-aminothymol, which compound was needed in connection with another study, the writers became interested in certain aspects of the chemical behavior of *p*-nitrosothymol and *p*-aminothymol. The present investigation represents an effort to facilitate the reduction of *p*-nitrosothymol to *p*-aminothymol and to learn something more of the chemical behavior of both compounds.

THEORETICAL AND EXPERIMENTAL

The flow sheet below indicates the reactions which received the most attention and shows graphically the compounds which resulted from these reactions

Tautomerism of p-Nitrosothymol.—The *p*-nitrosothymol (I) used in these studies was prepared in excellent yields by the method described by Kremers and Wakeman (1) in "Organic Syntheses." The compound was obtained as a buff-colored powder which was recrystallized from benzol in the form of practically white, short needles melting at 162° C. (uncorr.). *p*-Nitrosothymol was found to repel water so effectively that even on prolonged tritura-



Flow sheet

tion with aqueous liquids the compound could not be wetted. It was slowly but completely volatile with steam.

p-Nitrosothymol is freely soluble in pyridine and in solutions of the hydroxides of alkali metals producing in both instances a very dark brown solution. By the careful addition of hydrochloric acid the color of the dark brown solution was discharged and the light-colored *p*-nitrosothymol reprecipitated. These color changes may be explained on the basis that the practically colorless *p*-nitrosothymol obtained by the action of nitrous acid on thymol is tautomeric with the more highly colored thymoquinonemonoxime (Ib) which is obtained by condensing hydroxylamine with thymoquinone. The product in each case may be represented by the tautomeric equilibrium (I $\rightleftharpoons$ Ib). It is generally agreed that the nitroso-benzenoid structure prevails in a neutral medium whereas the oxime-quinonoid form predominates in alkaline and acid solutions (2). The intense brown color of the alkaline solution is presumably due to the anions from the highly ionized salt (II) formed from thymoquinonemonoxime (Ib) and the alkali (3). It is interesting to note that either a 0.15% aqueous solution of calcium hydroxide or a 20% aqueous solution of sodium carbonate is capable of effecting this tautomeric transformation while a 20% aqueous solution of sodium bicarbonate was without effect in this respect.

Hydrolysis of Thymoquinonemonoxime.—Although thymoquinone (III) may be obtained by oxidizing *p*-aminothymol (V) with ferric chloride (4) and by a number of other methods described in the literature (5), (6) it is still listed at 55 cents a Gm. (7).

Since the readily available *p*-nitrosothymol (I) is a tautomer of thymoquinonemonoxime (Ib) it seemed worth while to determine, whether it would be possible to bring about the tautomeric rearrangement of *p*-nitrosothymol into its quinonoid-oxime modification and then effect the hydrolysis of the oxime thereby directly obtaining thymoquinone (III). The following factors indicated that thymoquinone could be prepared by these reactions. (1) An acid medium would tend to convert the tautomeric compound to its quinonoid-oxime form (8). (2) The chemical behavior of the quinones indicate that the 6-membered ring has lost its aromaticity and is essentially an unsaturated diketone (9). (3) The monoximes of ketones may often be hydrolyzed by aqueous acids to yield the original ketone and hydroxylamine (10).

Recently Tseng, *et al.* (11), (12), claim to have obtained good yields of thymoquinone and phenan thraquinone by hydrolyzing thymoquinonemonoxime and phenanthraquinonemonoxime, respectively, by the use of 8% hydrochloric acid and in the case of the latter compound with the addition of formaldehyde as a catalyst. The results of Tseng, *et al.*, could not be reproduced with thymoquinonemonoxime although their procedure was followed and in one trial formaldehyde was used for its catalytic effect. It was observed during these reactions that the admixture of the *p*-nitrosothymol with the acid was very poor even after 15 hours of refluxing. Finally, with the knowledge that certain organic solvents facilitated the admixture of *p*-nitrosothymol with aqueous liquids and upon the assumption that the carbonyl group in formaldehyde was responsible for its catalytic property in hydrolyzing ketoximes this effort was made toward bringing about the desired reaction.

Three and a half grams (0.02 mole) of *p*-nitrosothymol was triturated with 2.5 cc. (0.04 mole) of acetone to form a paste. To this was added 50 cc. of 7% hydrochloric acid and the entire mixture was refluxed for twenty-four hours over an asbestos gauze by means of a direct flame. After about ten hours of refluxing the appearance of an appreciable quantity of yellow oil around the cooler portion of the boiling flask indicated that a fair amount of thymoquinonemonoxime had been hydrolyzed into thymoquinone. Steam distillation of the reaction mixture yielded 1.2 Gm. of thymoquinone which was obtained in the form of orange-colored, plate-shaped crystals which without further purification melted at 45° C. (corr.). The melting point of thymoquinone is given in the literature as 45.5° C. (13). This yield represents 36% of the theoretical.

A sample of thymoquinonedioxime (IV) was prepared by treating *p*-nitrosothymol with hydroxylamine but an attempt to hydrolyze this compound by means of 2% hydrochloric acid was not successful.

Reduction of p-Nitrosothymol.—There are several methods described in the literature for the reduction of *p*-nitrosothymol to *p*-aminothymol (14), (15). However, only one attempt to use catalytic hydrogenation for this purpose has been reported (16) and in this instance only about half the theoretical amount of *p*-aminothymol was obtained. The production of *p*-aminothymol by the catalytic reduction of *p*-nitrosothymol was therefore undertaken.

Following the procedure of Ott and Schroter (17) a palladium catalyst was prepared by shaking 0.05 Gm. of palladous chloride with 2 Gm. of animal charcoal in 100 cc. of distilled water in an atmosphere of hydrogen until no more hydrogen was absorbed. Eighteen grams (0.1 mole) of *p*-nitrosothymol were dissolved in 350 cc. of absolute ethyl alcohol by mechanical agitation. Dry hydrogen chloride was passed into the solution until 4 Gm. or 1.1 equivalents was absorbed. The palladiumized charcoal was added to the solution of *p*-nitrosothymol and the entire mixture was placed in a 500-cc. round-bottom flask which was attached by means of a ground glass connection to a hydrogen delivery train, assembled according to the directions of Hartung (18). Hydrogen was led into the flask through a large graduated cylinder and supplied throughout the reaction at a pressure of approximately 20 inches of water. Mechanical agitation of the flask was begun and continued until the

calculated quantity, 4500 cc. (0.2 mole), of hydrogen had been absorbed which usually required about four hours. The catalyst was filtered off by suction and the alcohol evaporated from the filtrate by means of a steam-bath. The *p*-aminothymol hydrochloride thus obtained was colored but after being washed by suction with several small portions of ethyl acetate a practically white compound resulted which weighed 19.5 Gm. representing 97% of the theoretical yield. (In another experiment, using a different type of hydrogenation apparatus, the Adams platinum catalyst (19) was found to be equally as efficient for this particular reduction.) The *p*-aminothymol hydrochloride melted at 252° C. (uncorr.) and could be obtained as extremely fine, needle-shaped, white crystals by dissolving in ethyl alcohol and allowing solvent to evaporate spontaneously.

Analyses:

Calculated for $C_{10}H_{16}ONCl$ Cl = 17.60
Found Cl = 17.90

This determination was made by the well-known Parr bomb method.

Unless at least an equivalent of hydrogen chloride was present to convert the freshly reduced *p*-aminothymol to its hydrochloride a considerable amount of oxidation took place before the compound could be isolated which resulted in the formation of a relatively large amount of a purple compound possessing the properties of a dye. This not only decreased the yield of the desired compound but made purification most difficult. The formation of the purple dye may be accounted for by assuming that the first product formed by the oxidation of *p*-aminothymol is the thymoquinone monoimine (VIII) which condenses with the unoxidized *p*-aminothymol (V) to form the anil (IX) which might be termed an indothymol. This explanation is supported by an experiment of Schwob (20) in which it was found that charcoal catalyzes the oxidation of a mixture of an aromatic diamine and a phenol to the corresponding indophenol and moreover the oxidation of *p*-aminothymol took place in absolute ethyl alcohol, an ideal medium for anil formation. The indothymol-like compound dissolved in alcohol to give a purple solution which was decolorized by the addition of hydrogen sulfide or ascorbic acid (21). The purple alcoholic solution was susceptible to color changes on the addition of an acid or a base (22). These properties of the purple compound likewise bear out its relationship to the indophenols.

Diazotization of p-Aminothymol.—An attempt to diazotize *p*-aminothymol was deemed worth while since the resulting diazonium salt, by virtue of its replaceable diazo group, would constitute an intermediate from which it might be possible to prepare medicinally important derivatives of thymol. It was found that attempts to replace the diazo group in the thymoldiazonium chloride (VI) by the usual method of warming the aqueous solution resulted in the formation of a red tar from which only a small amount of thymohydroquinone (VII) could be isolated. Since the formation of the highly colored tar is presumably due to a coupling reaction the method finally used to replace the diazo group with a hydroxyl group was based on the observation of Zeigler, *et al.* (23), that the chance of inter-molecular reaction is considerably diminished if the solution of the reacting substances is made sufficiently dilute.

Two grams (0.01 mole) of *p*-aminothymol hydrochloride were added to 50 cc. of water containing 4 cc. of concentrated hydrochloric acid. The mixture was cooled by means of an ice-bath to 3° C. and to it was slowly added under constant stirring 0.69 Gm. (0.01 mole) of sodium nitrite previously dissolved in 10 cc. water. As the nitrite solution was added the undissolved *p*-aminothymol hydrochloride disappeared and a brown foam began to appear on the surface of the solution. The foam soon became a dark-colored flocculent precipitate which was removed by filtration and discarded since its weight was found to be negligible. After removing the brown precipitate, the green solution of the diazonium salt was poured into 2 L. of strongly agitated boiling water. The solution was allowed to cool to room temperature and then evaporated under reduced pressure to about 1/20 of its original volume. The solution was cooled to 0° C. whereupon a crop of short, needle-shaped, yellowish crystals of thymohydroquinone precipitated. These crystals melted at 138° C. (uncorr.) and weighed 0.7 Gm. which represented a yield of 43% of the theoretical.

SUMMARY

1. The tautomerism of the *p*-nitrosothymol–thymoquinonemonoxime system has been studied with a view toward determining the effect of this on the hydrolysis of the compound.

2. Thymoquinone was obtained in fair yields by hydrolyzing *p*-nitrosothymol by means of a catalyst in an acid solution.

3. *p*-Nitrosothymol was quantitatively reduced to *p*-aminothymol by the use of hydrogen in the presence of a palladium catalyst and in a different experiment by the use of a platinum catalyst.

4. An explanation for the color changes involved in the oxidation of *p*-aminothymol has been offered and supported.

5. *p*-Aminothymol was diazotized and thymohydroquinone was obtained from the resulting diazonium salt by means of a new method.

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The Glycosides of Asclepias Cornuti or the Common Milkweed*

By A. E. Rihn† and H. G. DeKay‡

The common milkweed has been studied for its caoutchouc content (1), fibers, seed hairs, seed oil (2) and constituents in its lactiferous sap (3). A number of papers on these topics have been published since 1910.

The common milkweed was official in the U. S. P. from 1820 to 1864. The related species, *A. tuberosa* was official in the U. S. P. until 1916 when it was placed in the N. F. In 1936, it was deleted from the N. F.

Both species are members of the genus, *Asclepias*, and belong to the *Asclepiadaceæ* family. The common species has two botanical names, *Asclepias syriaca* (Linné, 1753) and *Asclepias Cornuti* (Decaisne, 1844). It may be differentiated from other species by its erect follicles which are covered with short soft processes and mounted on recurved pedicles. The leaf is broad and rounded at the apex, and different in other minor respects (4).

The sugars of the plant were studied by Gerhardt (6).

The related species native to Europe had been studied as far back as 1825 (5). Harnack (7), Gram (8), Tanret (9), Kubler (10) studied the glycoside from *Vincetoxicum officinale*. Gram also studied the glycosides from *A. curassavica* and *A. tuberosa* and found that they possessed a similar pharmacological activity.

Kubler assigned the formula, $C_{46}H_{70}(OCH_3)_4O_{16}$, to the glycoside obtained from *V. officinale*. It has since been determined that two forms of the compound exist, one water-soluble and the other water-insoluble.

Masson (11) isolated a saponid from *V. officinale* and found that it resembled the glycoside, vincetoxin. Van Rijn (12) states that vincetoxin is apparently identical with asclepiadin, the compound obtained from *A. tuberosa* and *A. curassavica* by Gram. A number of bulletins of the United States Department of Agriculture report the isolation of toxic gycosides from species of *Asclepias* native to the West.

Asclepias Cornuti has been used in dropsy, and as an emetic and cathartic. Gram's study on the pharmacological action of the glycoside showed that it affected the respiration, causing the heart to stop in diastole. No report of a thorough study on the glycosides of *A. Cornuti* was found in the literature.

EXPERIMENTAL

STUDIES ON ASCLEPIAS CORNUTI

The plants were gathered in Tippecanoe County,

* An abstract of a thesis presented to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Master of Science.

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Indiana, during August and September 1936. They were removed from the ground with rhizome and stem intact, and dried in the open air. After separation, the rhizome and stems were separately ground to a coarse powder, and when needed for treatment, were reduced to a finer powder in a hand mill. The powdered drug was first extracted with benzin to remove caoutchouc and other fatty material.

As a result of handling the fresh plants, an urticaria appeared. This affection was characterized by vesiculation and swelling of the hands, and persisted for approximately three weeks. The intense burning itch was alleviated by immersion in hot water, followed by application of Calamine Lotion.

In the processes of purification of the plant extracts, all evaporations were carried out *in vacuo*. The containing vessel was immersed in a water-bath at temperatures below 35° to 40° C. to hasten evaporation under reduced pressure.

The aqueous and alcoholic extracts of the plant left thick syrupy residues when evaporated to low volumes. Sucrose and glucose were identified in these residues. The timed osazone reaction given in Mulliken Volume I (13) was employed. It was found difficult to remove the glycoside with solvents in the presence of the hydrated sugars, since the sugars tended to form colloidal solutions in chloroform and alcohol.

STUDIES ON THE GLYCOSIDES

Part 1. Leaves and Stems

Experiment I.—The drug was percolated with lime water, the percolate acidified with acetic acid and filtered. The filtrate was treated with fuller's earth, which was collected on a filter paper, washed, dried and extracted with absolute alcohol. The bitter substance obtained did not show the properties of a glycoside.

Experiment II.—The drug was extracted with alcohol by percolation. The percolate was filtered, concentrated *in vacuo*, and allowed to evaporate spontaneously in the air until a heavy green precipitate had formed. The mixture was filtered, and the filtrate shaken out with ether. The other layer was evaporated and the residue dissolved in water and alcohol, and purified by treatment with lead acetate. The purified solution left a bitter residue which was non-glycosidal, and showed no properties of an alkaloid or tannin.

Experiments III and IV.—The drug was percolated with alcohol, and the extract mixed with magnesium oxide and evaporated to dryness. The residue was treated with absolute alcohol, and the solution purified by the lead acetate method of Rosenthaler. The filtrate obtained in this procedure was evaporated to dryness, redissolved in alcohol, and one volume of ether added to precipitate the sugars. After filtration, the filtrate was evaporated to dryness, and the residue extracted with warm distilled water. The mixture was filtered through talc on a Buchner funnel. The filtrate was colloidal and left a brown-colored residue which had the following properties:

1. It dissolved in water, giving a bitter, nauseating solution.
2. It was rendered insoluble by acids, but redissolved on addition of alkali.
3. It had no reducing power.
4. It gave negative tests with Mayer's reagent and tannic acid solution.
5. It was toxic to the common sparrow when injected into the abdominal cavity.

The residue which was not dissolved by warm water was removed from the talc by treatment with absolute alcohol. It possessed similar properties, but was non-toxic to the common sparrow.

The common sparrow was used as the test specimen in these experiments for two reasons: *first*, the isolation of the toxic substance was not accomplished until late in the year and it was impossible to obtain frogs and other accepted animals before completing the work; and *second*, we desired some subject that had similar ability of emesis as that of the human.

Inasmuch as this is the first time, so far as we know, that the sparrow has served for experimental purposes, the results may be open to question.

The observations upon the test bird were compared in each case with a control. The symptoms and results led us to conclude that the substance isolated was toxic.

Part 2. Glycoside of the Rhizome

Experiments I to IV.—As an introductory study, these experiments were conducted on a commercial preparation of *A. tuberosa*, "Asclepidin" (Parke, Davis Co., Detroit). Several glycosidal preparations were obtained, using the general method employed in the treatment of *Asclepias Cornuti* (cf. Exp. V). These products were similar in properties to asclepiadin, but varied in their toxicity when tested on the common sparrow.

The yield of the impure glycoside was approximately 0.001%.

Experiment V.—In this experiment, a glycoside was obtained from the rhizome of *Asclepias Cornuti*. The powdered drug was percolated with alcohol, and the percolate evaporated to low volume, filtered, and then evaporated to dryness in presence of magnesium oxide. The dried residue was extracted with alcohol, and the solution obtained filtered with the aid of talc. Sufficient water was added to the filtrate to make an 80% alcoholic solution, and this was purified by the lead acetate process. The purified solution was evaporated to drynes and extracted with absolute alcohol. The alcoholic solution was evaporated and the residue again extracted with absolute alcohol. Chloroform was added and the mixture filtered with the aid of talc. One volume of ether was added to three volumes of the filtrate, the mixture filtered, and the filtrate evaporated to dryness. The residue was dissolved in

chloroform, treated with activated charcoal and filtered through a charcoal mat on a Buchner funnel. The filtrate was evaporated to dryness and extracted with ether to remove impurities. The residue not dissolved by the ether was taken up in chloroform and the solution washed with several small portions of water. The chloroform was removed *in vacuo*, the residue obtained was dissolved in a known quantity of alcohol. Two volumes of ether were then added, the mixture shaken out with water and the aqueous layer filtered into a small dish. This was placed in a vacuum desiccator and evaporated to complete dryness. The transparent residue possessed the following properties:

1. It was tan in color.
2. It dissolved readily in water, alcohol and chloroform, but not in ether.
3. When hydrolyzed by boiling with dilute sulfuric acid, it reduced Fehling's solution. The untreated residue had no reducing power.
4. The solid and its solution possessed a bitter taste.
5. Mayer's reagent gave a negative text.
6. Tannic acid produced a copious white precipitate.
7. It showed the following actions on being heated:

Softening	94–100° C.
Fusion	107–108° C.
Swelled	120° C.
Darkened	160–180° C.
Reddish brown	203–205° C.

The heated mass was dissolved in water, and the solution tested. The substance no longer showed the properties of a carbohydrate.

8. After standing for eight days in a stoppered vial, it exhibited the same properties as before and was found to be toxic to sparrows.

The yield of the glycoside was approximately 0.002%.

In several other experiments, the glycoside was precipitated by addition of tannic acid solution to a purified extract of the rhizome. The precipitate was collected and treated with litharge. The dried mass was then extracted with absolute alcohol but no glycoside was obtained. Hence, it is apparent that this method should be used with caution.

CONCLUSIONS

1. Sucrose and glucose were present in the aqueous and alcoholic extracts obtained from parts of *A. Cornuti*.

2. The leaves and stems contain a bitter principle which may be divided into a toxic and a non-toxic fraction.

3. The leaves and stems examined did not contain any significant amount of glycosides.

4. A toxic glycoside which resembled asclepiadin in properties was isolated from the rhizome of *A. Cornuti*, apparently in impure form.

5. The overground portions of *A. Cornuti* contained a principle which was irritating to the dermis.

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Studies on Cantharides. I. The Titration of Cantharidin*,†

By Benjamin P. Hecht and Lloyd M. Parks‡

INTRODUCTION

For many centuries the drug Cantharides, commonly referred to as Spanish or Russian Flies, has been used in medicine. Its history dates at least as far back as the time of the early Greeks, as shown in the writings of Hippocrates.

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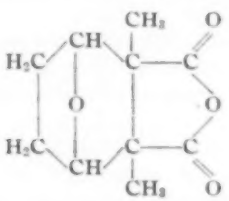
In medicine, the principle action of Cantharides is that of a vesicatory and the various uses to which it has been applied depend, more or less, upon this effect. The therapeutic action of the drug has been shown to depend upon the chief constituent, cantharidin, which was first isolated in 1810 by Robiquet (1). The chemistry of this substance has been developed by Picard (2), Homolka (3), Anderlini (4), Spiegel (5), Meyer (6), Danckwortt (7), Gadamer (8) and Rudolph (9). The following accepted structure has been advanced for cantharidin:



Since the efficacy of the drug depends entirely upon this active principle, it has been necessary to determine the content of cantharidin in evaluating Cantharides. Unfortunately, the methods that have been developed thus far have not proved to be satisfactory. These methods have all depended upon the separation of cantharidin in pure form and weighing the amount thus obtained. The present assay process of the U. S. Pharmacopœia, Eleventh Revision, which is of such nature, has several disadvantages. It gives results which are not accurate, for in the final step of the process, the cantharidin is not obtained in the pure state, but is always associated with inert material which is not easily removed. The process is also unusually long and time consuming.

Since cantharidin has been shown by Homolka (3), Danckwortt (7) and Gadamer (8) to be the anhydride of a dibasic acid, it seemed possible and practicable to determine it by acidimetric titration. The analysis of the di-methyl ester of cantharidin by Homolka, and of the potassium salt by Danckwortt, indicated a dibasic anhydride structure, the neutralization of which takes place according to the following equation:

$$C_{10}H_{12}O_4 + 2M'OH = C_{10}H_{12}O_5M_2' + H_2O$$

Upon reviewing the literature, it was found that cantharidin could not be titrated quantitatively. In this connection, Scoville (10), (11) has published the results of his unsuccessful attempts to titrate cantharidin acidimetrically, from which he concluded that the compound does not combine with alkali in any definite proportion during titration.

The discrepancy involved in the titration of cantharidin has been explained by Gadamer (12). He believed it to be due to hydrolysis of the salt formed, and has laid down the following system:

$$C_{10}H_{12}O_5K_2 + 2\ H_2O = C_{10}H_{12}O_5H_2 + 2\ KOH$$
$$KOH = K^+ + OH^-$$

Accordingly, the presence of excess hydroxyl ions account for an end-point before all of the anhydride can be titrated. The same explanation has been advanced by Danckwortt.

Danckwortt (7) has observed that about $1^1/_2$ carboxy groups can be accounted for in the titration of cantharidin. It is significant to note that Mulliken (14) does not include a neutralization equivalent for cantharidin.

From the above, it may be seen that any approach to a titrimetric method for the estimation of cantharidin in Cantharides must first be concerned with the titration of pure cantharidin itself. Once this is accomplished satisfactorily, the results may be applied to the drug and a satisfactory assay process may be developed.

EXPERIMENTAL

Inasmuch as it had been concluded from the work of Danckwortt (7), Scoville, (10), (11) and Gadamer (12) that cantharidin cannot be titrated quantitatively, it seemed desirable to repeat some of their experiments.

A quantity of cantharidin was obtained from S. B. Penick and Company. It had a melting point of 210–212° (uncor.), appeared in granular form and on careful examination exhibited a yellow color. The product was therefore purified by recrystallizing it from hot chloroform. Colorless, tabular prismatic and hexagonal-shaped crystals were obtained, M. P. 214–214.5° (uncor.).

DIRECT TITRATION OF CANTHARIDIN

In carrying out titrations of cantharidin, it was necessary to employ a solvent such as alcohol or acetone because of the insolubility of this compound in water and even in solutions of alkali, unless heated for some time.

With Aqueous Potassium Hydroxide.—A 0.1500-Gm. sample in neutral acetone solution gave no definite end-point when titrated with aqueous alkali, using phenolphthalein indicator. Addition of the aqueous alkali precipitated the cantharidin from acetone solution.

With Alcoholic Potassium Hydroxide.—To prevent the precipitation of cantharidin, alcoholic potassium hydroxide was used and the above experiment repeated. 0.1000 Gm. of cantharidin required about 6.4 cc. of $\frac{N}{10}$ alcoholic KOH, as compared to 10.2 cc., theoretical. The end-point was very indefinite.

RESIDUAL TITRATION OF CANTHARIDIN

In Acetone Solution.—Cantharidin was dissolved in neutral acetone, an excess of $\frac{N}{10}$ alcoholic KOH added, and the solution then titrated to neutrality with $\frac{N}{10}$ HCl, using phenolphthalein as indicator.

Sample	Gm. Cantharidin	Cc. $\frac{N}{10}$ KOH Required	Cc. $\frac{N}{10}$ KOH Calc.	% Deviation
1	0.1000	6.85	10.2	32.84
2	0.1000	8.6	10.2	15.68

This method gave a very sharp end-point, but the results were low and differed greatly in the two titrations.

In Alcoholic Solution.—The above experiment was repeated, with the cantharidin in alcohol solution.

Sample	Gm. Cantharidin	Cc. $\frac{N}{10}$ KOH Required	Cc. $\frac{N}{10}$ KOH Calc.	% Deviation
1	0.1000	6.8	10.2	33.35
2	0.1000	6.4	10.2	37.25

Again, the end-point in these titrations was sharp but the results remained low. These low results suggested that neutralization was incomplete, possibly because of the resistance which the anhydride structure offered to the action of alkali.

RESIDUAL TITRATION OF CANTHARIDIN AFTER REFLUXING WITH ALKALI

With Alcoholic Alkali.—In order to overcome the resistance of the anhydride structure to the action of alkali, which was suspected from the above experiments, heat was applied.

0.1000 Gm. of cantharidin was dissolved in 25 cc. of neutral acetone, 25 cc. of $\frac{N}{10}$ alcoholic KOH added, and the solution refluxed for $^1/_2$ hour. The solution was then cooled, and the excess alkali titrated with $\frac{N}{10}$ HCl, using phenolphthalein as indicator.

0.1000 Gm. cantharidin required 6.5 cc. of $\frac{N}{10}$ KOH as compared to 10.2 cc., theoretical. Per cent deviation, 36.27.

With Aqueous Alkali.—Cantharidin was refluxed with an excess of $\frac{N}{10}$ aqueous KOH until solution was effected and the excess alkali then titrated with $\frac{N}{10}$ HCl, using phenolphthalein as indicator.

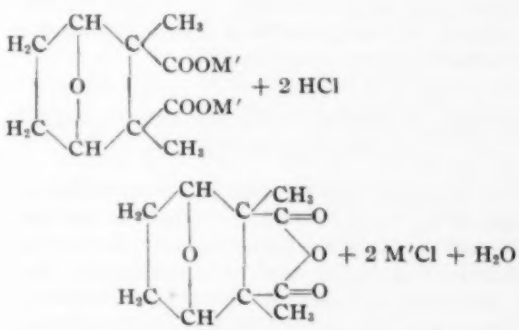
Sample	Gm. Cantharidin	Cc. $\frac{N}{10}$ KOH Required	Cc. $\frac{N}{10}$ KOH Calc.	% Error
1	0.1684	17.4	17.18	1.27
2	0.1751	17.8	17.86	0.33
3	0.2153	22.1	21.96	0.47

It required about an hour's refluxing before the solution of cantharidin resulted, the time varying with the fineness of the cantharidin.

The results of the first of these experiments verified the work of Scoville (10). The results of the latter experiment, however, showed that cantharidin can be titrated quantitatively in the absence of organic solvents, and indicated that the presence of such solvents, or, perhaps, the absence of water, are factors involved in the neutralization of the anhydride structure by alkali.

RESIDUAL TITRATION OF CANTHARIDIN, USING A WEAK ACID

It is well known that the addition of mineral acids to salts of cantharidic acid, the dibasic acid resulting upon the hydration of the acid anhydride, causes decomposition of these salts with the formation of cantharidin, the dibasic acid being quite unstable:



That this might explain the failure to titrate cantharidin was held plausible. Accordingly, a residual titration was tried, in which hydrochloric acid was replaced by a weak acid, potassium acid phthalate. A 0.1000-Gm. sample required 9.0 cc. of $\frac{N}{10}$ KOH as compared to 10.2 cc. theoretical. This low value indicated that decomposition of the salt does not occur during the residual titration of cantharidin when using strong mineral acids.

EFFECT OF STRONG ACIDS ON SALTS OF CANTHARIDIC ACID

There was the alternate possibility, in the above experiment, that potassium acid phthalate, a weak acid in the ordinary sense, was strong enough, on the other hand, to decompose the salts of cantharidic acid formed during titration. To investigate this possibility new experiments were devised to determine the effect of strong acids on the salts of cantharidic acid. The behavior of cantharidates toward strong acids is essentially that of a buffer action. If these salts performed as buffers during residual titrations, the low values experienced would become apparent.

Barium cantharidate and potassium cantharidate were prepared by neutralizing cantharidin with hot solutions of barium hydroxide and potassium hydroxide, respectively, and purifying the resulting salts. To 10 cc. of $\frac{N}{10}$ $Ba(OH)_2$ a quantity of barium cantharidate was added and the mixture titrated to neutrality with $\frac{N}{10}$ HCl, using methyl orange. The experiment was repeated with the addition of potassium cantharidate to 10 cc. of $\frac{N}{10}$ KOH. In each case 10 cc. of $\frac{N}{10}$ HCl were required for the titrations.

In these titrations the amount of acid required was equivalent in each instance to the amount of alkali originally used. This demonstrated that the presence of salts of cantharidic acid, formed during the residual titration of cantharidin, does not interfere with the process. Therefore, the buffer action of cantharidates is not a factor and does not explain the low results obtained in the titrations thus far. These experiments also showed that a strong mineral acid, such as hydrochloric, may be employed in residual titrations of cantharidin.

ATTEMPT AT RESIDUAL TITRATION OF CANTHARIDIN, USING BARIUM HYDROXIDE

It appeared thus far that the explanation advanced by Gadamer (12) (hydrolysis of the salt formed) to explain the failure to titrate cantharidin quantitatively, might have some foundation. But if this were the case, the neutralization could be made to go to completion by using barium hydroxide as the base. Since barium cantharidate is insoluble in water, the reversible reaction, and hydrolysis of the salt, would be prevented in accordance with the Law of Mass Action. Accordingly, this possibility was thought worthy of investigation. It was attempted to use barium hydroxide in the residual titration of cantharidin in alcohol solution, using a hot plate to keep the substances in solution. However, it was impossible to obtain a definite end-point and further work using barium hydroxide as the alkali was abandoned.

DETERMINATION OF THE p_H OF POTASSIUM CANTHARIDATE SOLUTIONS

A number of aqueous solutions of potassium cantharidate of various molar strengths were prepared and the p_H of these determined by means of a Cameron p_H Meter, using a glass electrode. The readings were made at a temperature of 25°. The results were as follows:

Strength of Potassium Cantharidate	p_H
$M/20$	10.25
$M/200$	10.20
$M/400$	10.00
$M/800$	9.85
$M/1600$	9.60
$M/2000$	9.58

By determining these p_H values several facts were revealed which ultimately led to a better understanding of the problem.

First, phenolphthalein was shown to be a correct indicator to use in the titration of cantharidin when a strong base is employed, as the salt had a p_H of 10 ($M/400$). Thymolphthalein is also a suitable indicator, and perhaps to be preferred.

Indicator	p_H Range	Transition
Phenolphthalein (0.003M)	8.3–10	9.3
Thymolphthalein	9.5–10.5	9.5

Second, some idea as to the extent of hydrolysis was obtained. Because of certain properties of cantharidin the experimental determination of the degree of hydrolysis was made difficult. In carrying out such a measurement, it was first necessary to determine the dissociation constant of cantharidic acid, the hydrate of cantharidin. This acid is, however, unstable and cannot be isolated, the addition of strong acids to solutions of the salt causing decomposition with the precipitation of cantharidin. However, by finding the p_H of potassium cantharidate, an approximation of the dissociation constant of this acid was obtained.

Relatively, cantharidic acid was shown to be stronger than boric acid but weaker than hydrosulfuric acid. This was demonstrated by adding acids of different dissociation constants to a solution of potassium cantharidate, and observing if precipitation of cantharidin occurred.

Relative Strength of Cantharidic Acid

Acid Added to Solution of Potassium Cantharidate	Dissociation Constant of Ac		Precipitation of Cantharidin Observed
Hydrochloric	.....		+
Acetic	1.86×10^{-5}	25°	+
Propionic	1.4×10^{-5}	25°	+
Butyric	1.53×10^{-5}	25°	+
Caproic	1.43×10^{-5}	18°	+
Hydrosulfuric	5.7×10^{-8}	18°	+
Cantharidic	.....?		..
Boric	6.6×10^{-10}	25°	−

The first five acids were added directly to the solution of the salt; hydrosulfuric was added by passing H_2S through the solution for several hours; boric acid was added by first dissolving it in distilled water.

From the p_H data, a more definite value for the dissociation constant of cantharidic acid was obtained. The $M/200$ solution of potassium cantharidate, corresponding to 0.01 normal, had a p_H of 10.2. According to the data of Bjerrum (13), this value lies between 4 and 3 ($H^{-14} - H^{-10.2} = H^{-3.8}$). By interpolation, the dissociation constant of cantharidic was calculated to be $10^{-8.4}$, in round numbers. In the same manner a p_H of 9.58, found for the $M/2000$ solution, corresponded to $10^{-8.2}$ as the dissociation constant. Taking the mean of these two values, the dissociation constant of cantharidic acid was placed at $10^{-8.3}$, equivalent to 5×10^{-9}.

It was now possible to calculate the per cent of hydrolysis of the salt, since there exists a mathematical relationship between hydrolysis of the salt and the dissociation constant of the acid. Since the latter value was found to be greater than 10^{-6}, the degree of hydrolysis is more than 1% and the following mathematical expression is applicable:

$$\frac{CX^2}{1 - X} = \frac{Kw}{Ka} = Kh$$

where

C = molar concentration of the salt
Kw = ion product of water
Ka = dissociation constant of the acid
X = degree of hydrolysis

Substituting, using 1.27×10^{-14} as the value of the ion product of water at 25°,

$$\frac{0.005X^2}{1 - X} = \frac{1.27 \times 10^{-14}}{5 \times 10^{-9}}$$

solving,

$$X = 0.0228$$

The degree of hydrolysis of potassium cantharidate in $M/200$ concentration at 25° is therefore 2.28%. While this value found for X may not be entirely accurate, it is, nevertheless, a close approximation.

In the titration of cantharidin, any error involved due to hydrolysis of the salt should be in the order of 2%. By proper choice of an indicator, such as phenolphthalein, this error can be overcome or at least much reduced. Actually, the magnitude of error experienced was much greater, although the per cent of deviation varied considerably.

Per Cent Deviation

1 33.35 (in alcohol)
2 37.25 (in alcohol)
3 32.84 (in acetone)
4 15.68 (in acetone)

RESIDUAL TITRATION OF CANTHARIDIN WITH REMOVAL OF ORGANIC SOLVENT

That the alcohol or acetone which had to be used as a solvent for cantharidin must have some effect on the titration results was strongly indicated by the results of previous experiments. These solvents have low dielectric constants and may greatly influence ionization. Since neutralization depends upon H^+ ions and OH^- ions furnished by acid and base, respectively, depression of the ionization of either would account for anomalies in titrations. In spite of the fact that alcohol affects the ionization of fatty acids, these acids can still be titrated in alcohol solution. With the idea in mind that the ionization of cantharidin is greatly depressed by these organic solvents, a method was devised whereby they could be removed.

Cantharidin was dissolved in 25 cc. of neutral acetone, followed by an excess of $N/10$ alcoholic KOH, and 25 cc. of distilled water. The solution was then evaporated until the acetone and alcohol were removed, leaving an aqueous solution of potassium cantharidate and an excess of potassium hydroxide. The excess alkali was then titrated with $N/10$ HCl, using phenolphthalein. A correction was applied by running a blank for the acetone. The following results were obtained:

Sample	Gm. Cantharidin	Cc. $\frac{N}{10}$ KOH Required	Cc. $\frac{N}{10}$ KOH Calculated	% Error
1	0.0949	9.5	9.68	−1.85
2	0.0857	8.5	8.74	−2.74
3	0.0567	5.9	5.78	+2.07
4	0.0700	7.4	7.14	+2.77
5	0.0800	8.1	8.15	−0.61
6	0.1522	15.5	15.53	−0.19
7	0.2007	20.4	20.47	−0.34

By this method of titration the results were quantitative, within the limits of experimental error. The removal of the alcohol and acetone, which had to be used originally to dissolve the cantharidin, permitted complete neutralization. This showed fairly conclusively that the failure to titrate cantharidin by any of the methods hitherto used was due to a depression of the ionization of cantharidin by alcohol, acetone or benzene, or to the absence of water as a hydrating medium. As a result there was required a greater amount of H^+ ions which had to be supplied by the acid used in the residual titration, and this in turn caused the low results always experienced. Additional evidence that the presence of these organic solvents prevented the quantitative titration of cantharidin had already been furnished by the results of a previous experiment on the residual titration of cantharidin after refluxing with aqueous alkali, in which, in the absence of organic solvents, quantitative results were obtained.

STUDY OF THE TITRATION OF OTHER WATER INSOLUBLE ANHYDRIDES

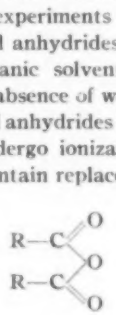
Inasmuch as cantharidin is an acid anhydride, insoluble in water, it was thought that other water-insoluble acid anhydrides should behave similarly upon titration. Accordingly, a study was undertaken to determine the effect of alcohol on the titration of two other water-insoluble acid anhydrides, benzoic and phthalic acid anhydrides.

RESIDUAL TITRATION OF BENZOIC AND PHTHALIC ANHYDRIDES IN ALCOHOL

Benzoic and phthalic acid anhydrides were each dissolved in alcohol, followed by an excess of $N/10$ alcoholic KOH. The excess alkali was titrated with $N/10$ HCl, using phenolphthalein. A blank was run for the alcohol.

			Cc. $\frac{N}{10}$ KOH		
Sample	Anhydride	Gm.	Required	Calculated	% Error
1	Phthalic	0.2049	22.2	27.65	19.53
2	Phthalic	0.4413	36.6	59.63	38.58
3	Benzoic	0.2019	11.7	17.85	34.45
4	Benzoic	0.3034	21.1	26.85	24.41

From the above data the low results obtained are comparable to those obtained in the similar titrations of cantharidin. Thus the depression of the ionization of cantharidin is not due to some property exclusively inherent in its structure, but is common to other water-insoluble acid anhydrides as well. These experiments demonstrate that water-insoluble acid anhydrides, in general, cannot be titrated in organic solvents, such as alcohol and acetone in the absence of water. Furthermore, the structure of acid anhydrides is such that it should be expected to undergo ionization with difficulty, since it does not contain replaceable hydrogen:



This is entirely compatible with the fact that in alcohol or a similar nonionized solvent, ionization is made even more difficult. Organic solvents unquestionably affect the indicators as well, but the extent to which this occurs is not comparable to the effect which it exerts on the water-insoluble acid anhydrides.

RESIDUAL TITRATION OF BENZOIC AND PHTHALIC ANHYDRIDES AFTER REFLUXING WITH AQUEOUS ALKALI

This experiment was carried out in a similar manner to that used for cantharidin in a previous experiment. The anhydrides were refluxed with an excess of $N/10$ aqueous KOH until solution was affected and the excess alkali then determined by titration with $N/10$ HCl, using phenolphthalein.

			Cc. $\frac{N}{10}$ KOH		
Sample	Anhydride	Gm.	Required	Calculated	% Error
1	Phthalic	0.3012	41.1	40.70	−1.47
2	Phthalic	0.2017	28.2	27.98	−0.71
3	Phthalic	0.3110	43.5	43.37	+0.29
4	Benzoic	0.2002	17.8	17.71	+0.56
5	Benzoic	0.2952	26.2	26.12	+0.35

These results, as in the case of cantharidin, are quantitative within the limits of experimental error, and again emphasize the point that water-insoluble acid anhydrides are titratable, but not in the exclusive presence of organic solvents.

RESIDUAL TITRATION OF BENZOIC AND PHTHALIC ANHYDRIDES WITH REMOVAL OF ORGANIC SOLVENT

The method used here was the same as that used for cantharidin in a previous experiment. The anhydride was dissolved in 25 cc. of alcohol, followed by an excess of $N/20$ alcoholic KOH, and 25 cc. of distilled water, and the solution evaporated to a volume of about 10 cc., or until the alcohol was no longer detectable. The excess alkali was then titrated with $N/10$ HCl, using phenolphthalein. A blank was run for the alcohol used.

			Cc. $\frac{N}{20}$ KOH		
Sample	Anhydride	Gm.	Required	Calculated	% Error
1	Phthalic	0.2906	78.1	78.54	−0.56
2	Phthalic	0.1681	45.6	45.43	+0.37
3	Benzoic	0.2008	34.8	35.52	+2.02
4	Benzoic	0.2004	35.6	35.46	+0.39

The results obtained here, as in the similar experiment, are again quantitative. This method of titrating water-insoluble acid anhydrides would be especially useful for determining the neutralization equivalents or for the quantitative estimation of those acid anhydrides which are water insoluble and which may be decomposed by the heat of refluxing, or are volatile.

SUMMARY AND CONCLUSIONS

1. The results of other investigators, namely, Scoville, Gadamer and Danckwortt, in their attempts to titrate cantharidin quantitatively in the presence of such organic solvents as alcohol, acetone or benzene, have been confirmed.

2. The failure to titrate cantharidin in the presence of organic solvents has been shown to be due, not to hydrolysis of the salt formed, as advanced by Gadamer, or to the buffer action of the salt formed, or to the action of strong mineral acids on the salt formed; it has been shown to be due to some effect exerted by the organic solvent used to

dissolve the cantharidin. This effect is apparently a depression of the ionization or neutralization of cantharidin, due possibly to the low dielectric constant of the solvent and its inability to ionize.

3. The effect of alcohol on other water-insoluble acid anhydrides has been investigated and it was shown that the two studied, benzoic and phthalic anhydrides, behaved similarly to cantharidin in attempts to titrate them quantitatively in the presence of alcohol. The effect of alcohol is thus apparently not restricted to cantharidin, but is general for all water-insoluble acid anhydrides.

4. A method has been developed for the quantitative titration of cantharidin, in which the organic solvent used to dissolve the compound is removed completely during the procedure, in the presence of water and excess alkali. This method yielded equally satisfactory results for cantharidin and the other anhydrides, benzoic and phthalic, and is generally applicable to all water-insoluble acid anhydrides.

5. The p_H's of solutions of potassium salts of cantharidic acid of various molar strengths have been determined. From these it was possible to estimate the ionization constant of the free acid, which was calculated as being 5×10^{-9}, stronger than boric acid but weaker than hydrosulfuric acid. It was also possible to calculate the degree of hydrolysis of the potassium salt as being approximately 2%.

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The Sterol and Resin Alcohols from Celastrus Scandens

*By Ole Gisvold**

During the investigation of the pigments contained in the petroleum ether extract of the outer bark of the root of *Celastrus Scandens*, it was deemed advisable to investigate the phytosterols which might be present. In addition to the sterol that was apparently a sitosterol or a mixture of sitosterols, three resin alcohols were isolated.

EXPERIMENTAL

Phytosterol.—The pigment-free petroleum ether extract obtained from the outer bark of the root of *Celastrus Scandens* was saponified with alcoholic potassium hydroxide and the nonsaponifiable residue extracted by means of a continuous extraction apparatus with Skelly-solve B. The solvent was removed on a steam-bath and the residue taken up in alcohol. Fractional crystallization enabled the separation of some phytosterol. The residual sterol was isolated by means of its digitonide (1). The digitonide was decomposed by precipitating the digitonin with ether from a pyridine solution of the complex (2).

The purified sterol melted at 137.5° C. and its acetate at 121° C. It had a specific rotation of −32.5 in chloroform at 25° C. It gave the color reactions characteristic of sterols.

A number of sterols have been reported in the literature having physical constants closely approaching those given above. It is highly possible that it is a mixture. The amount isolated was too small to warrant further investigation.

Resin Alcohol No. 1.—The sterol-free nonsaponifiable material upon spontaneous concentration deposited needle-like crystals in an oily residue. The oily material was successfully removed by washing with cold Skelly-solve B. The crystals purified by several recrystallizations from Skelly-solve B became soft at 215° C. and clear at 221° C.

These crystals gave a deep purple color when subjected to the Lieberman-Burchard test. The material available was too small for further investigation.

Resin Alcohol No. 2.—The mother liquor upon spontaneous evaporation left a very viscous oil from which needle-like crystals slowly separated.

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These crystals were removed from the noncrystalline portion by stirring with Skelly-solve B in which the latter was readily soluble. The crystalline portion was removed by a suction filter. This material when purified by several recrystallizations from a mixture of alcohol and ether, gave rosettes of needles. The crystals melted at 175° C. and its acetate at 195° C. The free alcohol gave a rose to rose purple and finally a deep purple when subjected to the Lieberman-Burchard test for sterols.

Resin Alcohol No. 3.—Prolonged extraction of the nonsaponifiable portion with Skelly-solve B gave a very small amount of material which crystallized in rosettes of needles from the concentrated extract. Several crystallizations from alcohol and ether gave rosettes of platelets. These crystals although appearing to be very pure gave a very indefinite melting point. They appeared to soften at about 220° C. and liquefied at 235° C.

These crystals gave a pink to orange to a red-orange with the Lieberman-Burchard reagent. The amount of material was too small to warrant the preparation of a derivative or further investigation.

SUMMARY

A phytosterol, apparently one of the sitosterols or a mixture of sterols, and three resin alcohols were isolated from the nonsaponifiable portion of the petroleum ether extract of the outer bark of the root of *Celastrus Scandens*.

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Incompatibilities in Prescriptions

III. The Use of Inert Powders in Capsules to Prevent Liquefaction Due to Formation of a Eutectic Mixture*,†

By William J. Husa‡ and Charles H. Becker

In filling prescriptions for powder mixtures to be dispensed in capsules, pharmacists frequently encounter difficulties due to liquefaction or formation of a pasty mass (1), (2). The present paper is devoted to the correction of incompatibilities due to formation of a mixture having a melting point below room temperature (eutectic mixture).

One of the methods used in dealing with powders which liquefy or become pasty is to incorporate an inert powder such as starch, magnesium oxide, magnesium carbonate, talc, lactose, etc. In spite of the wide use of such inert powders, apparently no systematic studies have previously been made to determine the relative efficiency of the various powders, or to ascertain the best methods of combining the inert powders with the ingredients prescribed.

The present investigation was carried out to supply practicing pharmacists and teachers of pharmaceutical dispensing with more exact information regarding the best methods of selecting and using inert powders to overcome liquefaction in prescriptions for capsules.

EXPERIMENTAL PART

Prescription No. 1

℞ Camphor	gr. 1/4
Salol	gr. iii

Fac tales capsulæ no. xx. One capsule t. i. d. a. c.

When the ingredients of this prescription were triturated a pasty mass was formed. A study was made to determine the relative efficiencies of eight different inert powders in preventing or obviating the difficulty. In each case the camphor was triturated with the inert powder; the salol was then added and the mixture triturated lightly. The temperature at the time of compounding was from 70° to 74° F. The finished capsules were kept under observation for two weeks in open beakers as well as in closed capsule vials.

With reference to the period of observation, it may be said that many pharmacists lay too much stress on the appearance of capsules at the time they are dispensed, with little consideration of the changes which may occur before all the capsules are used.

Obviously the use of an inert powder increases the size of the capsule to a certain extent depending on the relative quantity of powder used and its bulkiness. This factor must always be kept in mind since many patients find it difficult to swallow the larger capsules. In exceptional cases it may be necessary to divide the material into twice the number of capsules designated and to double the dose

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† This paper is based on part of a thesis presented to the Graduate Council of the University of Florida by Charles H. Becker, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

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as to number of capsules. In the present study the size of capsule required in each case was recorded and the results have a bearing on the choice of the quantity and kind of inert powder that would be best to use.

In all the tables in the present paper the following abbreviations are used: L. = soft mass or liquid; P. = dry powder; D. = damp powder; S. D. = slightly damp powder; C. M. = cement-like mass. The quantity of inert powder is stated in grains per capsule.

Table I.—Prescription No. 1. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	2	D.	L.	L.	L.	L.
2	1	S. D.	D.	D.	L.	L.
3	1	P.	P.	P.	S. D.	D.
4	0	P.	P.	P.	S. D.	D.
5	0	P.	P.	P.	S. D.	D.
Light Magnesium Oxide						
1	2	P.	L.	L.	L.	C. M.
2	1	P.	D.	D.	L.	C. M.
3	0	P.	P.	P.	P.	S. D.
4	00	P.	P.	P.	P.	S. D.
5	00	P.	P.	P.	P.	S. D.
Talc						
1	2	L.	L.	L.	L.	L.
2	1	D.	L.	L.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	D.	D.	D.
5	0	S. D.	S. D.	D.	D.	D.
Lactose						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	L.	L.	L.
3	1	S. D.	D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	D.	D.
5	0	S. D.	S. D.	S. D.	D.	D.
Magnesium Carbonate						
1	2	P.	P.	P.	P.	P.
2	1	P.	P.	P.	P.	P.
3	0	P.	P.	P.	P.	P.
4	00	P.	P.	P.	P.	P.
5	00	P.	P.	P.	P.	P.
Corn Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	L.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	D.	D.
5	0	S. D.	S. D.	S. D.	S. D.	D.
Wheat Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	L.	L.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	S. D.	D.
5	0	S. D.	S. D.	S. D.	S. D.	D.

Table I.—(*Continued*)

Potato Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	L.	L.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	D.	D.
5	0	S. D.	S. D.	S. D.	S. D.	D.

Table I shows that magnesium carbonate was most effective in preventing liquefaction, one gr. per capsule being sufficient to maintain the ingredients as a powder. Light magnesium oxide was somewhat better than heavy magnesium oxide but both of these powders were less efficient than magnesium carbonate. Talc, lactose and the starches were rather ineffective. It was observed that all capsules containing light magnesium oxide developed a pink color, which was more intense with the smaller proportions of light magnesium oxide. With the use of one or two grs. of light magnesium oxide per capsule, the ingredients eventually formed a mass of cement-like hardness. In all cases of liquefaction or pastiness, the ingredients shrunk to about half of the original volume. The results with the capsules stored in closed vials were similar to those with the capsules placed in open beakers; for this prescription, tight closure of the container was of no distinct advantage.

Since various textbooks have recommended that starch should be dried at 100° C. for use in powders, experiments were carried out to determine whether or not such dried starch had an advantage over ordinary starch. Similar comparisons were made with the other inert powders used in the present study. In each case, a portion of the powder was dried to constant weight at 100° C. The percentage loss in weight on drying was as follows: potato starch, 15.1; corn starch, 12.0; wheat starch, 11.7; magnesium carbonate, 2.6; heavy magnesium oxide, 1.5; light magnesium oxide, 1.0; talc, 0.2; lactose, 0.1.

Prescription No. 1 was filled using the same procedure as before, except that the oven-dried inert powders were used; the results are given in Table II.

Table II.—Prescription No. 1. With Oven-Dried Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	2	D.	L.	L.	L.	L.
2	1	P.	S. D.	D.	D.	D.
3	1	P.	P.	P.	P.	S. D.
4	0	P.	P.	P.	P.	S. D.
5	0	P.	P.	P.	P.	S. D.

Table II.—(*Continued*)

Light Magnesium Oxide						
1	2	P.	L.	L.	L.	C. M.
2	1	P.	P.	S. D.	S. D.	S. D.
3	0	P.	P.	P.	P.	S. D.
4	00	P.	P.	P.	P.	S. D.
5	00	P.	P.	P.	P.	S. D.
Talc						
1	2	D.	L.	L.	L.	L.
2	1	S. D.	L.	L.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	D.	D.	D.
5	0	S. D.	S. D.	D.	D.	D.
Lactose						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	D.	L.	L.
3	1	S. D.	S. D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	D.	D.
5	0	S. D.	S. D.	S. D.	D.	D.
Magnesium Carbonate						
1	2	P.	P.	P.	P.	P.
2	1	P.	P.	P.	P.	P.
3	0	P.	P.	P.	P.	P.
4	00	P.	P.	P.	P.	P.
5	00	P.	P.	P.	P.	P.
Corn Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	L.	L.	L.
3	1	S. D.	D.	D.	D.	D.
4	1	P.	S. D.	S. D.	D.	D.
5	0	P.	S. D.	S. D.	D.	D.
Wheat Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	L.	L.	L.
3	1	S. D.	D.	D.	D.	D.
4	1	S. D.	S. D.	S. D.	D.	D.
5	0	P.	S. D.	S. D.	D.	D.
Potato Starch						
1	2	L.	L.	L.	L.	L.
2	1	D.	D.	L.	L.	L.
3	1	S. D.	D.	D.	D.	D.
4	1	P.	S. D.	S. D.	D.	D.
5	0	P.	S. D.	S. D.	D.	D.

Table II again indicates that magnesium carbonate was most effective in preventing liquefaction. Drying to constant weight was of practically no advantage in the case of talc, lactose and the various starches. Drying was slightly beneficial for light and heavy magnesium oxides. Results on the capsules stored in closed vials were similar to the results on capsules stored in open containers as given in Table II.

It would be expected that the condition of the air as to temperature and humidity might have an effect on the stability of capsules in certain cases. The capsules of the previous experiment which had not shown liquefaction of the contents after two weeks were tested for stability at higher humidity by storage under a bell jar in which the air had previously been adjusted to a relative humidity of 75. The results showed that this humidity had no effect on the contents of the capsules in open beakers after three days. However, the gelatin capsules themselves became damp and soft. After reëxposure to the atmosphere the capsules regained their original condition after about a half day with the exception that the contents of the capsules containing light or heavy magnesium oxide became as hard as cement. There was no change in the capsules in closed vials.

In compounding prescriptions containing substances which tend to liquefy, it is usually considered best to mix one of the ingredients with an inert powder before mixing with the remaining substances or to mix each ingredient separately with some of the inert powder before combining the various ingredients. However, it has also been suggested (3) that the inert powder should be added to the liquefied material in a mortar. To determine the effect of variations in the method of compounding, prescription No. 1, with the addition of one gr. of magnesium carbonate per capsule, was compounded in several different ways as follows: (A) the salol and magnesium carbonate were triturated and the camphor added with light trituration, (B) same as A except that heavy trituration was employed, (C) the camphor and magnesium carbonate were triturated and the salol added with light trituration, (D) same as C except that heavy trituration was employed, (E) the camphor was triturated with part of the magnesium carbonate and the salol was triturated with the remainder of the magnesium carbonate and the two resulting powders were then mixed with light trituration, (F) same as E except that heavy trituration was employed, (G) the camphor, previously powdered with aid of a few drops of alcohol, was mixed with the magnesium carbonate and then the salol was added with light trituration, (H) the salol and camphor were triturated to form a pasty mass, the magnesium carbonate was added and the mixture triturated lightly, (I) same as H except that the pasty mass was added to the inert powder.

Table III.—Prescription No. 1. Effect of Variations in Method of Compounding. Capsules Stored in Open Containers

Method	Time in Days 0	1	2	4	14
A	P.	P.	P.	P.	P.
B	S. D.	S. D.	S. D.	S. D.	D.
C	P.	P.	P.	P.	P.
D	S. D.	S. D.	S. D.	S. D.	D
E	P.	P.	P.	P.	P.
F	S. D.	S. D.	S. D.	S. D.	D.
G	P.	P.	P.	P.	P.
H	S. D.	S. D.	S. D.	S. D.	S. D.
I	S. D.	S. D.	S. D.	S. D.	S. D.

The results in Table III show that it is unwise to triturate the liquefiable ingredients before adding the inert powder. It is preferable to first mix one incompatible ingredient with the inert powder or each incompatible ingredient with a separate portion of the inert powder. The results also clearly indicate that light trituration may be used but that heavy trituration is objectionable.

Tests were made to determine whether camphor might be lost from the capsules on standing due to its volatility. Prescription No. 1 was compounded using one gr. of heavy magnesium oxide per capsule in one test and using the same proportion of lactose in another test. Each capsule was weighed accurately on an analytical balance. The capsules were then stored for three weeks in open containers and also in closed vials. Although the contents of the capsules liquefied in each case, there was no appreciable gain or loss in weight of the capsules in either type of container.

Prescription No. 2

℞ Camphor	gr. i
Salol	grs. iii
Aspirin	grs. ii
Caffeine citrate	gr. i

M. ft. of such doses, capsules no. xii.

On compounding the prescription as written, a damp powder results, gradually becoming semi-liquid. To overcome the formation of a pasty mass, it was recommended (4) that half of the citrated caffeine be replaced by half its weight of caffeine base (in order to adjust the strength), and that one gr. of heavy magnesium oxide to the capsule be added, the latter to be combined with each ingredient separately before mixing all to form a uniform powder.

Table IV.—Prescription No. 2. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days: 0	1	2	4	14
Heavy Magnesium Oxide						
1	1	D.	L.	L.	C. M.	C. M.
2	0	P.	L.	C. M.	C. M.	C. M.
3	00	P.	C. M.	C. M.	C. M.	C. M.
4	00	P.	C. M.	C. M.	C. M.	C. M.
5	00	P.	C. M.	C. M.	C. M.	C. M.
Light Magnesium Oxide						
1	0	P.	L.	L.	C. M.	C. M.
2	00	P.	P.	D.	D.	C. M.
3	00	P.	P.	S. D.	S. D.	C. M.
4	0[a]	P.	P.	P.	P.	C. M.
5	0[a]	P.	P.	P.	P.	C. M.
Magnesium Carbonate						
1	0	P.	D.	D.	L.	L.
2	0[a]	P.	S. D.	S. D.	S. D.	L.
3	0[a]	P.	P.	P.	P.	P.
4[a]	0	P.	P.	P.	P.	P.
5[a]	0	P.	P.	P.	P.	P.

[a] Double the number of capsules were made.

Table IV.—(*Continued*)

Talc						
3	00	P.	L.	L.	L.	L.
Lactose						
5	00	P.	L.	L.	L.	L.
Dried Corn Starch						
5	00	P.	L.	L.	L.	L.
Dried Wheat Starch						
5	00	P.	L.	L.	L.	L.
Dried Potato Starch						
5	00	P.	L.	L.	L.	L.

On combining the ingredients as advised and placing individual doses in No. 1 capsules, the contents liquefied within a day. After standing four days the ingredients developed into a mass of cement-like hardness. Similar results were obtained when using 2 grs. of the inert powder per capsule and placing individual doses in No. 0 capsules. Using 3, 4 and 5 grs. of the inert powder, respectively, per individual dose and using No. 00 capsules, liquefaction did not occur, but the ingredients developed into a mass of cement-like hardness.

Tests were carried out in which prescription No. 2 was filled, with the addition of inert powders. The temperature was between 75° and 78° F. In each case the camphor was triturated with the inert powder and the aspirin, citrated caffeine and salol were added in the order named, after which the final mixture was triturated lightly.

The results show that magnesium carbonate was most efficient as an inert powder. Although as much as 3 grs. of the inert powder per capsule was required to prevent liquefaction, it did not change the ingredients into a mass of cement-like hardness. Both light and heavy magnesium oxides were equally as efficient as magnesium carbonate in preventing liquefaction but the capsules containing these inert powders developed into a cement-like mass after standing two weeks. Talc, lactose and the various dried starches did not prevent liquefaction even when using 5 grs. of the inert powder per capsule. In all capsules where liquefaction or a soft mass developed, the ingredients shrunk considerably.

The appearance of the capsules placed in closed containers was similar to those placed in open beakers and neither type of container had any distinct advantage over the other.

To determine the effect of variations in the method of compounding, prescription No. 2, with the addition of three grs. of magnesium carbonate per capsule, was compounded in several different ways as follows: (A) the salol and magnesium carbonate were triturated first and the camphor, citrated caffeine and aspirin were added in the order named,

the entire mixture being triturated lightly, (B) same as A except that the final mixture was triturated with pressure, (C) the camphor and magnesium carbonate were triturated first and the citrated caffeine, aspirin and salol were added in the order named, the final mixture being triturated lightly, (D) same as C except that the final mixture was triturated with pressure, (E) part of the magnesium carbonate was mixed with the camphor and the remainder of the magnesium carbonate was mixed with the salol; the resulting powders were mixed lightly and the citrated caffeine and aspirin were added after which the entire mixture was triturated lightly, (F) same as E except that the entire mixture was triturated with pressure, (G) the camphor, previously powdered with the aid of a few drops of alcohol, was mixed with the magnesium carbonate and the citrated caffeine, aspirin and salol were added in the order named and the final mixture was triturated lightly, (H) the ingredients were triturated until the mixture liquefied, the magnesium carbonate was added and the entire mixture was triturated, (I) same as H except that the liquid mixture was added to the magnesium carbonate.

Table V.—Prescription No. 2. Effect of Variations in Method of Compounding

Capsules Stored in Open Containers

Method	Time in Days 0	1	2	4	14
A	P.	P.	P.	P.	P.
B	S. D.	D.	D.	D.	D.
C	P.	P.	P.	P.	P.
D	S. D.	D.	D.	D.	D.
E	P.	P.	P.	P.	P.
F	S. D.	D.	D.	D.	D.
G	P.	P.	P.	P.	P.
H	S. D.	S. D.	S. D.	S. D.	S. D.
I	S. D.	S. D.	S. D.	S. D.	S. D.

The results in Table V clearly indicate that it is preferable to first mix one incompatible ingredient with the inert powder or each incompatible ingredient with a separate portion of inert powder. It is shown that heavy trituration is objectionable. The results on capsules stored in closed vials were similar to those on capsules placed in open containers.

Prescription No. 3

℞ Acetophenetidin	gr. i
Acetylsalicylic acid	gr. ii
Quinine ethylcarbonate	gr. ii

M. ft. caps. No. 1. Disp. tales no. xii.

On mixing acetylsalicylic acid and quinine ethylcarbonate a dry powder resulted. However, the ingredients developed into a mass of cement-like hardness within a day. After standing two weeks, the ingredients shrank to one-half of the original size and remained in a hardened condition. A mixture of acetophenetidin and quinine ethylcarbonate remained dry after standing two weeks. There was no hardening of the contents. On compounding the prescription as written a dry powder resulted and individual doses were put in No. 1 capsules. The ingredients developed into a cement-like mass within a day and, after standing two weeks, the contents shrank and remained in a hardened condition.

Prescription No. 3 was filled with the addition of inert powders at a temperature of 75° to 78° F. In each case the quinine ethylcarbonate was triturated with the inert powder and then the acetylsalicylic acid and acetophenetidin were added in the order named.

Table VI.—Prescription No. 3. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	0	P.	P.	P.	P.	P.
Light Magnesium Oxide						
1	0	P.	P.	P.	P.	P.
Talc						
1	0	P.	C. M.	C. M.	C. M.	C. M.
Lactose						
1	0	P.	C. M.	C. M.	C. M.	C. M.
Magnesium Carbonate						
1	0	P.	P.	P.	C. M.	C. M.
Dried Corn Starch						
1	0	P.	C. M.	C. M.	C. M.	C. M.
3	0	P.	C. M.	C. M.	C. M.	C. M.
Dried Wheat Starch						
1	0	P.	C. M.	C. M.	C. M.	C. M.
Dried Potato Starch						
1	0	P.	C. M.	C. M.	C. M.	C. M.

Table VI shows that best results were obtained with light and heavy magnesium oxides, which in this particular case did not form cement-like masses. The other inert powders prevented liquefaction but the ingredients formed into hard, solid bodies. Similar results were obtained for capsules stored in closed vials.

To ascertain whether tight packing of the ingredients in capsules had any part in developing the contents into a cement-like mass, the prescription was filled using 1 gr. of magnesium carbonate per capsule and individual doses were placed in some capsules tightly and in others loosely. After standing four days, the contents of the tightly packed capsules were of cement-like hardness while the contents of the loosely packed capsules remained in the same condition as when filled.

Prescription No. 4

℞	Phenyl salicylate	gr. xxxvi
	Acetophenetidin	
	Quinine hydrochloride aa	gr. xxiv
	Camphor	gr. x
	Mild mercurous chloride	gr. iss

M. Fiat cap. no. xxiv.

This prescription when compounded as written develops into a pasty mass.

Prescription No. 4 was filled with the addition of inert powders, the temperature being 78° to 80° F. In each case the camphor was triturated with the inert powder and the mild mercurous chloride, quinine hydrochloride, acetophenetidin and phenyl salicylate were added in the order named; the final mixture was triturated lightly.

Table VII.—Prescription No. 4. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	1	S. D.	C. M.	C. M.	C. M.	C. M.
2	0	P.	C. M.	C. M.	C. M.	C. M.
3	0	P.	C. M.	C. M.	C. M.	C. M.
Light Magnesium Oxide						
1	0	P.	C. M.	C. M.	C. M.	C. M.
2	0	P.	P.	P.	P.	P.
3	00	P.	P.	P.	P.	P.
Magnesium Carbonate						
1	0	P.	D.	D.	L.	L.
2	0	P.	P.	P	P.	P.
3	00	P.	P.	P.	P.	P.
Talc						
5	0	P.	D.	L.	L.	L.
Lactose						
5	0	S. D.	L.	L.	L.	L.
Dried Corn Starch						
5	0	S. D.	L.	L.	L.	L.
Dried Wheat Starch						
5	0	S. D.	L.	L.	L.	L.
Dried Potato Starch						
5	0	S. D.	L.	L.	L.	L.

Table VII shows that magnesium carbonate and light magnesium oxide gave the best results. Talc, lactose and the various dried starches did not prevent liquefaction. Heavy magnesium oxide prevented liquefaction but the contents of the capsules became cement-like masses. Similar results were obtained for capsules kept in closed vials.

Prescription No. 5

℞	Phenyl salicylate	℥ii
	Acetylsalicylic acid	℥ i
	Antipyrine	℥i
	Strychnine sulfate	gr. 1/4

Mix and make capsules No. 24.
Label: One every four hours.

As this prescription contains several liquefiable combinations, some tests were first made on pairs of ingredients. On mixing acetylsalicylic acid and antipyrine, a damp powder resulted which liquefied in capsules within a day. A mixture of acetylsalicylic acid and phenyl salicylate in capsules gave a slightly damp powder after standing for one week. Capsules containing phenyl salicylate and antipyrine liquefied after standing for one week.

Prescription No. 5 was filled with the addition of inert powders, the temperature being 78° to 80° F. In each case the antipyrine was first powdered and the inert powder added; the strychnine sulfate, phenyl salicylate and acetylsalicylic acid were then added in the order named and the final mixture was triturated lightly.

Table VIII.—Prescription No. 5. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	0	P.	C. M.	C. M.	C. M.	C. M.
2	00	P.	C. M.	C. M.	C. M.	C. M.
Light Magnesium Oxide						
1	0	P.	P.	P.	P.	P.
Magnesium Carbonate						
1	0	P.	L.	L.	L.	L.
2	00	P.	P.	P.	S. D.	L.
3[a]	0	P.	P.	P.	P.	P.
Talc						
5	00	P.	S. D.	L.	L.	L.
Lactose						
5	00	S. D.	L.	L.	L.	L.
Dried Corn Starch						
5	00	P.	L.	L.	L.	L.
Dried Wheat Starch						
5	00	P.	L.	L.	L.	L.
Dried Potato Starch						
5	00	P.	L.	L.	L.	L.

[a] Double the number of capsules were made.

The results in Table VIII show that light magnesium oxide was most effective in preventing liquefaction. Magnesium carbonate was efficient when

3 grs. of the inert powder per capsule were used. Heavy magnesium oxide prevented liquefaction, but capsules containing this inert powder developed the contents into a mass of cement-like hardness. Talc, lactose and the various dried starches did not prevent liquefaction even when using 5 grs. of the inert powder per capsule. Similar results were obtained on capsules stored in closed vials.

An experiment was carried out to determine whether pressure, such as applying weight on a thin layer of the mixed ingredients or packing the powders tightly in capsules would have any effect in hastening liquefaction. Prescription No. 5 was filled using 1 gr. of magnesium carbonate per capsule and the resulting powder was placed on a glass plate. A portion of the powder was spread out in a thin layer and a 1000 Gm. weight was placed over it. The other portion was also treated in the same way except that no weight was placed over it. Likewise, capsules were filled in which the ingredients were packed tightly in No. 0 capsules and packed loosely in No. 00 capsules. The capsules and spread powders were examined at one-hour intervals.

Table IX.—Prescription No. 5. Effect of Pressure

Time Intervals in Hours	Powder with Weight over It	Powder without Weight	Powder in 0 Capsules	Powder in 00 Capsules
0	P.	P.	P.	P.
1	S. D.	P.	S. D.	P.
2	S. D.	P.	S. D.	P.
3	D.	P.	D.	P.
4	D.	S. D.	D.	S. D.
5	D.	S. D.	D.	S. D.
6	L.	S. D.	L.	D.
10	L.	D.	L.	D.
12	L.	D.	L.	D.
13	L.	D.	L.	L.
14	L.	L.	L.	L.

The results in Table IX show that the application of pressure on the powder hastened liquefaction.

Prescription No. 6

℞ Acid Acetylsalicylic	℥ ii
Amidopyrine	℥ ii
Citrated Caffeine	℥ ss

Div. cap. no. xviii.

This prescription (5) yields a dry powder but after a few days the contents of the capsules become damp. When the citrated caffeine was replaced by half its weight of caffeine base a dry powder was obtained and individual doses were put in No. 00 capsules; the contents of the capsules became damp after one week.

Prescription No. 6 was filled with the addition of inert powders, the temperature being between 78° and 80° F. In each case the acetylsalicylic acid was triturated with the inert powder, the amidopyrine and citrated caffeine were added in the order named and the mixture was triturated lightly. Due to the fact that the addition of the inert powder increased the bulk, each dose was placed in two capsules rather than in one.

Table X.—Prescription No. 6. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Time in Days: 0	1	2	4	14
Heavy Magnesium Oxide					
1	P.	S. D.	D.	C. M.	C. M.
2	P.	C. M.	C. M.	C. M.	C. M.
3	P.	C. M.	C. M.	C. M.	C. M.
Light Magnesium Oxide					
1	P.	P.	P.	P.	D.
2	P.	P.	P.	P.	P.
3	P.	P.	P.	P.	P.
Talc					
1	P.	D.	D.	D.	D.
2	P.	S. D.	D.	D.	D.
3	P.	S. D.	S. D.	S. D.	D.
Lactose					
1	P.	D.	D.	D.	D.
2	P.	S. D.	D.	D.	D.
3	P.	S. D.	S. D.	S. D.	D.
Magnesium Carbonate					
1	P.	P.	P.	P.	D.
2	P.	P.	P.	P.	P.
3	P.	P.	P.	P.	P.
Dried Corn Starch					
1	P.	D.	D.	D.	D.
2	P.	S. D.	D.	D.	D.
3	P.	S. D.	S. D.	S. D.	D.
Dried Wheat Starch					
1	P.	D.	D.	D.	D.
2	P.	S. D.	D.	D.	D.
3	P.	S. D.	S. D.	S. D.	D.
Dried Potato Starch					
1	P.	D.	D.	D.	D.
2	P.	S. D.	D.	D.	D.
3	P.	S. D.	S. D.	S. D.	D.

Table X indicates that light magnesium oxide and magnesium carbonate prevented the formation of a damp powder. The contents of the capsules containing heavy magnesium oxide assumed a cement-like hardness. Talc, lactose and the various dried starches were ineffective. Similar results were obtained with capsules stored in closed vials.

Prescription No. 7

℞ Acetylsalicylic acid	gr. v
Methenamine	gr. v

Ft. caps. d. t. d. xii. no.

In filling this prescription a damp powder resulted; after one day the contents of the capsules liquefied and on longer standing a yellowish green color developed.

Prescription No. 7 was filled with the addition of inert powders. The temperature at the time of compounding was from 78° to 80° F. In each case the methenamine was mixed with the inert powder and the acetylsalicylic acid added, the final mixture being triturated lightly.

Table XI.—Prescription No. 7. With Inert Powders

Capsules Stored in Open Containers

Inert Powder, Grs.	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide					
1	P.	L.	L.	L.	C. M.
3	P.	D.	D.	C. M.	C. M.
5	P.	S. D.	C. M.	C. M.	C. M.
Light Magnesium Oxide					
1	P.	L.	L.	C. M.	C. M.
3	P.	D.	L.	C. M.	C. M.
5	P.	P.	D.	C. M.	C. M.
Talc					
1	P.	L.	L.	L.	L.
3	P.	L.	L.	L.	L.
5	P.	L.	L.	L.	L.
Lactose					
1	S. D.	L.	L.	L.	L.
3	P.	L.	L.	L.	L.
5	P.	L.	L.	L.	L.
Magnesium Carbonate					
1	P.	L.	L.	L.	L.
3	P.	D.	L.	L.	L.
5	P.	P.	L.	L.	L.
Dried Corn Starch					
1	P.	L.	L.	L.	L.
3	P.	L.	L.	L.	L.
5	P.	L.	L.	L.	L.
Dried Wheat Starch					
1	P.	L.	L.	L.	L.
3	P.	L.	L.	L.	L.
5	P.	L.	L.	L.	L.
Dried Potato Starch					
1	P.	L.	L.	L.	L.
3	P.	L.	L.	L.	L.
5	P.	L.	L.	L.	L.

Capsules Stored in Closed Vials

Heavy Magnesium Oxide					
1	P.	D.	C. M.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.
Light Magnesium Oxide					
1	P.	D.	C. M.	C. M.	C. M.
3	P.	P.	P.	P.	S. D.
5	P.	P.	P.	P.	P.
Talc					
1	P.	D.	D.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.
Lactose					
1	S. D.	D.	D.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.
Magnesium Carbonate					
1	P.	P.	P.	S. D.	L.
3	P.	P.	P.	P.	S. D.
5	P.	P.	P.	P.	P.
Dried Corn Starch					
1	P.	D.	D.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.
Dried Wheat Starch					
1	P.	D.	D.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.
Dried Potato Starch					
1	P.	D.	D.	C. M.	C. M.
3	P.	P.	C. M.	C. M.	C. M.
5	P.	C. M.	C. M.	C. M.	C. M.

Table XI shows that in this prescription the results were different according to whether the capsules were stored in open containers or in closed vials. In capsules stored in open containers light and heavy magnesium oxides caused the formation of cement-like masses and capsules containing light magnesium oxide assumed an orange color which was most intense when one or two grains of the inert powder were used. With other powders the contents liquefied.

In capsules stored in closed vials the use of talc, lactose, heavy magnesium oxide and the various starches caused formation of cement-like masses. Magnesium carbonate and light magnesium oxide in the proportion of three grains per capsule prevented liquefaction when the capsules were stored in closed vials.

DISCUSSION OF RESULTS

Inert powders which are light and fluffy appear to be most efficient in preventing liquefaction due to formation of a eutectic mixture. The results with various prescriptions indicate that magnesium carbon-

ate and light magnesium oxide are most effective while heavy magnesium oxide is less effective and talc, lactose and various starches are comparatively inefficient in preventing liquefaction. The probable explanation of the results is that powders which are light and fluffy are more effective in holding apart the particles of the substances which tend to liquefy on contact. When a light, fluffy powder is used it is somewhat more difficult to fill the capsules since the resulting powder packs less readily and has a tendency to fall out of the body of the capsule during the filling.

In some instances the use of light or heavy magnesium oxide caused the contents of the capsule to change into a hard, cement-like mass. Tests on some of the hardened masses showed that they did not disintegrate in water in four days; in 0.5 per cent solution of hydrochloric acid the solid mass was reduced slightly in size but retained its form. Since insoluble masses of this kind would probably pass through the alimentary tract without disintegrating it is important that the pharmacist take precautions to prevent their formation. In most cases cement-like masses did not form when magnesium carbonate was used as the inert powder.

SUMMARY

Experiments were carried out with various prescriptions for capsules to determine the relative efficiency of various inert powders in preventing liquefaction due to formation of a eutectic mixture. Inert powders which are light and fluffy appear to be most efficient. Magnesium carbonate and light magnesium oxide proved most effective, heavy magnesium oxide ranked next, and talc, lactose and various starches were least effective. In some cases, particularly with light and heavy magnesium oxide, the contents of the capsules assumed a cement-like hardness.

As to the various methods of compounding, the results show that it is better to first mix one incompatible ingredient with the inert powder or to mix each incompatible ingredient with a separate portion of the inert powder. Heavy trituration hastens liquefaction. Likewise the use of too small a capsule hastens liquefaction due to the tightness of packing.

Apparently exposure of the filled capsules to the air usually has little or no effect when the liquefaction is due to formation of a eutectic mixture.

In most cases the use of about two grs. of magnesium carbonate or light magnesium oxide per capsule stabilized the capsules over a period of two weeks.

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The Status of Phenol in Ointment of Phenol, U. S. P.*

By William A. Prout† and A. Clifton Smith, Jr.‡

To meet the United States Pharmacopœial requirements, Ointment of Phenol must contain not less than 1.8 per cent nor more than 2.2 per cent of C_6H_5OH. As is noted, a tolerance of 0.4 per cent is allowed to take care of possible error in weighing, questionable strength of the phenol used and probable loss during manufacture of the ointment. The purpose of this investigation was to determine whether or not various samples made by different individuals actually met the requirements as designated in the United States Pharmacopœia in respect to the content of phenol.

G. R. Page (1) of England, working on the British Pharmacopœia (1932) Ointment of

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Phenol, which should contain 3 per cent of the active constituent, found that the loss of phenol during preparation may be from 3.7 per cent to 5.2 per cent of that added, and that there was a further decrease on keeping, the amount varying with the conditions of storage. He found that ointments stored in earthenware containers at room temperature lost phenol rapidly, and that the strength fell approximately 33 per cent after two years' storage. Ointments stored in collapsible tubes, however, showed little or no loss of phenol after ten months. He attributed the loss of phenol to volatilization.

EXPERIMENTAL

Two ointments of phenol were prepared, one in accordance with the United States Pharmacopœia XI directions (2) and the other according to a modified method of procedure. Following are the two formulas and directions:

Ointment of Phenol, U. S. P.

Phenol	2 Gm.
Yellow Wax	5 Gm.
Petrolatum	93 Gm.
To make about	100 Gm.

Melt the yellow wax and phenol on a water-bath, add the petrolatum and stir the mixture until it congeals.

Ointment of Phenol, Modified Method

Phenol	2 Gm.
Yellow Wax	5 Gm.
Petrolatum	93 Gm.
To make about	100 Gm.

Melt the yellow wax on a water-bath, add the petrolatum and, when liquefied, remove the source of heat. Cool to about 70° C., add the phenol and stir the mixture until congealed.

In order to reproduce as nearly as possible the ordinary conditions of manufacture as carried out by the average pharmacist, small quantities (50 Gm.) of ointment were prepared by different individuals, using phenol, which had been previously assayed, and dispensing balances to weigh the ingredients. The ointments were then immediately assayed according to the Pharmacopœia (3), which directs that the phenol be separated from the ointment base by distillation with steam, and that the phenol in the distillate be estimated by use of Koppeschaar's Solution.

The results of the assays are shown in the following tables:

Table I.—Ointment of Phenol, U. S. P.

Sample Number	Phenol, C. P. (Baker's) Added	Found by Assay	Per Cent Loss of Phenol
1	2.0000	1.6395	18.02
2	2.0000	1.6892	15.54
3	2.0000	1.6405	17.97
4	2.0000	1.6582	17.09
5	2.0000	1.6281	18.59
6	2.0000	1.6842	15.79
7	2.0000	1.6482	17.59
8	2.0000	1.6354	18.23
9	2.0000	1.6784	16.08
10	2.0000	1.7244	13.78
11	2.0000	1.6982	15.09
12	2.0000	1.7102	14.46
13	2.0000	1.5984	20.08
14	2.0000	1.6386	18.07
15	2.0000	1.7236	13.82
16	2.0000	1.6280	18.60
17	2.0000	1.7423	12.88
18	2.0000	1.6832	15.84
19	2.0000	1.6204	18.98
20	2.0000	1.6674	16.63

Table II.—Ointment of Phenol, Modified Method

Sample Number	Phenol, C. P. (Baker's) Added	Found by Assay	Loss of Phenol
1	2.0000	1.9702	1.49
2	2.0000	1.9742	1.29
3	2.0000	1.9849	0.75
4	2.0000	1.9502	2.49
5	2.0000	1.9498	2.51
6	2.0000	1.9784	1.08
7	2.0000	1.9836	0.82
8	2.0000	1.9827	0.86
9	2.0000	1.9588	2.06
10	2.0000	1.9672	1.64
11	2.0000	1.8580	2.10
12	2.0000	1.9546	2.27
13	2.0000	1.9468	2.66
14	2.0000	1.9322	3.39
15	2.0000	1.9406	2.97
16	2.0000	1.9398	3.01
17	2.0000	1.9782	1.09
18	2.0000	1.9688	1.56
19	2.0000	1.9842	0.79
20	2.0000	1.9764	1.18

DISCUSSION

The results of this investigation have shown conclusively that there is a decided loss of phenol during the manufacture of the ointment when made as directed by the United States Pharmacopœia XI, while the loss of phenol was much smaller in the ointment made according to the modified procedure of manufacture. The average loss of phenol in the pharmacopœial ointment amounts to 16.65 per cent while the average

loss of phenol in the ointment made by the modified method was only 1.80 per cent.

While possible error in weighing, and improper mixing may also contribute to the loss of phenol, volatilization appears to be the outstanding factor responsible for so great a loss of phenol during the manufacture of the ointment.

Phenol (4), as described in the French Pharmacopœia, is slightly hygroscopic, slowly volatilized at room temperature and very rapidly volatilized at 100° C. As the United States Pharmacopœia directs that the yellow wax (5), which has a melting point of 62° C. to 65° C., and the phenol be melted on a water-bath, evidently the heat is great enough to volatilize some of the phenol before the remainder of the base is finally added.

CONCLUSIONS

1. Ointments manufactured in accordance with the United States Pharmacopœia directions lose phenol rapidly during preparation and the strength may fall to as low as 1.59 per cent.

2. Ointments of phenol when made according to a modified procedure show much less loss of phenol during the preparation. All samples of this series fell within the pharmacopœial limits of the active constituent.

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Notes on the Preparation of Lotio Flava, N. F. VI*

By Henry M. Burlage†

In a course dealing with the pharmacy of inorganic substances and preparations the writer has assigned, among other official preparations involving chemical reactions, Lotio Flava to be made by the students in the laboratory. It has been observed over a period of several years that a goodly number of the individual products handed in for grading showed, instead of the expectant yellow precipitate, a reddish brown one. This has occurred with such frequency that it could not be explained entirely on the basis of faulty technique by the individual worker and it was decided to attempt an explanation of this variation in the appearance of the product.

It was at first thought that the variation might be due to an inferior or impure mercuric chloride used in the formula. However, products from different manufacturers purchased at various times yielded similar results in the final product. One writer states that the reddish or brownish precipitate may be a basic compound which forms because the lime water was deficient in calcium hydroxide (hence too much $HgCl_2$) or too much mercuric chloride has been used (1). He further states that there is very little difference in the composition of the two oxides (?) formed. The yellow oxide is more finely divided and reflects the yellow rays of light and the red form is crystalline (hence of large size) and reflects the red rays of light (2).

EXPERIMENTAL

The following experiments were performed:

(1) The N. F. formula and procedure were followed using freshly prepared lime water *decanted* from the excess of calcium hydroxide.

(2) Repeat (1) using freshly prepared lime water *filtered*.

(3) Repeat (1) using freshly prepared lime water *shaken*.

(4) The N. F. procedure was employed using freshly prepared and filtered Liquor Calcis and 3.25 Gm. mercuric chloride per 1000 cc. (0.25 Gm. more than the N. F. formula).

(5) Same as (4) using 2.75 Gm. $HgCl_2$ per 1000 cc. (0.25 Gm. less than the N. F. formula).

(6) Same as (1) but using lime water that had been exposed to the air for several days, then filtered.

(7) Same as (2) after bubbling carbon dioxide through the lime water for a time.

(8) Same as (2) adding 0.1% sodium bicarbonate.

(9) Same as (2) using 0.3% sodium bicarbonate.

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† The author wishes to acknowledge the assistance of W. K. Minnick and P. L. Trotter on a portion of this work.

(10) Same as (2) introducing 0.5% sodium bicarbonate.

The results of these experiments are shown in the following table:

Table.—Nature of Precipitate in Lotio Flava

Experiment Number	Precipitate	Precipitate after Two Months
1	S	Coarse; settles quickly
2	S	Coarse; settles quickly
3	S	Finer; settles more slowly
4	S	Coarse; settles quickly
5	S	Finer; settles somewhat slower
6	RB	Fine; settles slowly
7	RB	Fine; settles slowly
8	S	Fine; settles slowly
9	S	Coarse; settles quickly
10	RB	Fine; settles slowly

Legend: S = satisfactory; RB = reddish brown.

CONCLUSIONS

An examination of the results shown in the above table and the various procedures would indicate that the preparation is sensitive to the *carbonate ion* and that the reddish brown precipitate appears when the lime water has been exposed to the air or when this ion (CO_3^{--}) has been introduced into the lime water by other means. This would indicate that a portion of the precipitate formed might be a basic carbonate or in other words, a mixture of the oxide and this compound. Our observations seem to refute the statement that the reddish brown precipitate is formed because of too much mercuric chloride or the use of lime water deficient in calcium hydroxide. It would seem fitting, however, to recommend that the National Formulary direct the use of freshly prepared lime water in the preparation of Lotio Flava.

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NOTICE

Papers to be presented at the next Annual Convention in Richmond, Va., May 5th–12th, should be sent to the respective Section Secretaries promptly.

M.-E. Chevreul. The Fiftieth Anniversary of His Death

By Mary Elvira Weeks and Lyle O. Amberg*

On April 9, 1889, the life of the great French chemist Michel-Eugène Chevreul came to a peaceful close shortly before his one hundred and third birthday. For nearly eighty years he had contributed article after article to the scientific journals. Both his parents had lived past the age of ninety years (1), (2), and one of his works was dedicated "to the memory of Michel Chevreul and of Etinnette-Madeleine Bachelier, respectful homage from their son in recognition of the moral sense and good health they transmitted to him" (1).

Chevreul was born at Angers on August 31, 1786, and at the age of seven years he looked through the window, with childlike curiosity, to watch the guillotining of two young girls. In later life, however, his bright outlook was not marred by the dark scenes of his childhood (2). He received his early training at the Central School in Angers. At the age of seventeen years he went to Paris to study under the great pharmacist and chemist Nicolas-Louis Vauquelin, assistant to A.-F. de Fourcroy at the Collège de France, and three years later he took charge of the laboratory. His first scientific memoir (3), a chemical examination of some fossil bones, was published in 1806, and in the same year he assisted Vauquelin in analyzing some human hair (4).

Since Vauquelin was greatly interested in the purple vapor evolved when indigo is heated, he asked Chevreul to investigate it (5). Chevreul found that indigo purified by successive treatments with water, alcohol and hydrochloric acid gave off "a vapor of a superb purple, much more intense than that produced by an equal quantity of the same indigo when not purified; from which it follows that this phenomenon is produced by the indigo and not by foreign bodies to which it is united." He showed that this vapor is the indigo itself, most of which volatilizes without decomposition; that indigo can be purified by sublimation or by recrystallization; that pure indigo is purple and not blue; and that the intensity of the color increases as the molecules are brought closer together. He also demonstrated the presence of indigo in its white, reduced form in pastel, or woad. Four years later he published a paper on the preparation of natural indigo (5). He also examined Brazilwood and logwood (6) and discovered brazilin and hæmatoxylin.

In 1823 he published the results of eleven years of research on the hardness of soaps (7). He found that the sodium soap of a given fat is harder (less soluble in cold water) than the corresponding potassium soap but that the fat as well as the alkali

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is a determining factor. He determined the solubility in cold water of the sodium and potassium soaps of stearic, "margaric" and oleic acids, and showed that the hard soaps lose water on exposure to air. He also proved that the hardest soaps are obtained from the fats in which the stearates are high in proportion to the "margarates" and oleates and that the soaps from natural oils can be imitated by mixing the fats to be saponified.

Chevreul found that when soaps are used as detergents, they are hydrolyzed to form free alkali and an acid salt, such as potassium bistearate of the approximate composition $C_{18}H_{35}O_2K.C_{18}H_{36}O_2$. His work was confirmed by Krafft and Stern in 1894, who it in separating the solid acids ("margaric," palmitic and stearic) from the liquid oleic acid, the potassium salt of which remained in solution. Although Chevreul's "margaric acid" was merely an intimate mixture of palmitic and stearic acids, a compound $C_{17}H_{34}O_2$, intermediate between palmitic and stearic acids, is now known by the same name.

Chevreul gave the name phocenic acid to the principal odorant (trivaleric acid) of the soaps of dolphin oil, "hircic acid" to that of mutton fat and butyric acid to the odorant of butter fat, and stated that the latter "contains two other acids which I name capric and caproic" (7), (11). His "hircic acid" is now known to be a mixture of homologous

M. Chevreul in His Hundredth Year at Work in His Laboratory. (From a Photograph by M. David.)

concluded that it would be difficult to add anything essentially new to "the simple and clear views of Chevreul, Berzelius and Persoz" (8).

In 1809 a specimen of soft soap used in fulling cloth was sent to Chevreul for analysis (9), (10). When he dissolved some of it in a large volume of water, he observed some shining crystals. "When one places some soap formed from pork fat and potash in a large volume of water," said he, "part of it dissolves, while another part precipitates in the form of little shining spangles, which I shall call nacreous matter. After having decanted off the liquor, one washes the deposit repeatedly with cold water and then throws it on a filter." He then washed the pearly scales with alcohol and, upon treating them with hydrochloric acid, liberated a new fatty acid which he named "margaric acid" because of its mother-of-pearl luster. He also prepared the same substance from other soaps and used fatty acids present in mutton suet. Chevreul made ultimate analyses of butyric, phocenic and "hircic" acids and emphasized the practical value of a knowledge of the odorous organic acids in the manufacture of cheese.

When C. M. d'Ohsson, a friend of Berzelius, and the physiologist François Magendie visited Chevreul, he showed them some of his new products, including butyric acid, "which smelled horribly bad . . . I tried in vain," said d'Ohsson in a letter to Berzelius "to discover some new instruments in his and Gay-Lussac's laboratories. I found, on the contrary, that everything there was of the greatest simplicity, and your laboratory certainly does not suffer by comparison" (12).

Even in his early thirties, Chevreul was already at the height of his powers. In 1819 Berzelius wrote to W. von Hisinger, "The greatest chemists now in France are Gay-Lussac, Dulong, Chevreul and

Thenard" (12). During his visit to Paris in the summer of 1819 Berzelius wrote, "With Thenard, Gay-Lussac, Dulong and Chevreul I lived on the same footing as among my old schoolmates at Upsala" (13). Berzelius often watched the experiments on saponification, and once said of Chevreul, "He is the most excellent observer of details; none of them are too small for his notice; and the services he has rendered to the science, which are by no means small, result merely from his study of details. However, he is not so fortunate in the presentation of general theoretical concepts . . . " (12), (14). Chevreul believed that "elementary and professional education should be confined to that which is true and easily demonstrated to be true" (15).

In 1813 he began to lecture on fats and their saponification products before the French Academy of Sciences, and ten years later these discourses were published under the title "Chemical researches on fatty substances of animal origin" (16), (17). Fats had formerly been regarded as acid substances which could unite directly with a base to form a soap, which was thus believed to be a binary salt of fat and alkali. In 1741 Claude-Joseph Geoffroy (Geoffroy the Younger), a member of a famous family of French apothecaries, showed that, when a soap solution is neutralized with a mineral acid, the resulting fatty substance differs from the original one by dissolving readily in alcohol.

"When olive oil," said he, "is separated, by means of an acid, from the soap which has been dissolved in spirits, it becomes similar to an essential oil; it is more flammable, gives less soot, and combines immediately with spirit of wine. On distillation, however, it does not, like the true essential oils, pass over with the water" (19).

In 1783 C. W. Scheele, in his quiet pharmacy, heated a mixture of olive oil and litharge and obtained a "sweet principle," which is now known as glycerol (18). He also obtained the same substance from other vegetable and animal fats and clearly distinguished it from sugar and honey.

In 1816 Chevreul quantitatively saponified the fats of man, the sheep, the cow, the jaguar and the goose, and found that, in each case, about ninety-five per cent of the fat had been saponified. He computed by difference that there must be about five per cent of soluble matter in each of these fats, and observed that "the sirupy liquid which contained the sweet principle produced by saponification (glycerol), although evaporated until it began to volatilize, always weighed much more than the fat had lost of soluble matter" (20). He believed at first that he had merely been unable to remove all the water and "saline matter." When in 1818 he discovered phocenin (glyceryl valerate) and butyrin, he realized that they were compounds of the volatile acids and anhydrous glycerol (20) and that the excess weight represented the quantity of water fixed by the fat to form the glycerol in saponification. He knew too that the fatty acids and the glycerol were not merely mixed in the fats, for, if that were true, it would be possible to leach out the glycerine with water. Moreover, alcohol, which dissolves both fatty acids and glycerin, does not dissolve the fat. He compared the fats in this respect with ethyl acetate and postulated that they must be formed of fatty acids and a substance which adds on water to form glycerol.

In the first book on his treatise on fats Chevreul showed that the classification, according to melting point, into waxes, fats, butters and oils was unscientific, and described methods for the ultimate analysis of fatty substances. Book two describes many fats and the saponification products obtained from them with different alkalis; an investigation of the fatty acids and their physical and chemical properties; a study of the metallic salts of oleic acid; and descriptions of cholesterin, ethal (cetyl alcohol), cetin, olein, phocenin, butyrin and "hircin" (17).

In the third book he discussed the saponification of cetin, a crystalline fat obtained from spermaceti. He obtained no glycerol from the cetin, but ethal (cetyl alcohol) instead. In book four he gave comparative results of the saponification of various fats, including those from dolphins and porpoises, from edible fish and from cadavers. Although Fourcroy had not distinguished clearly between the adipocere he discovered in dead bodies, the spermaceti from the sperm whale and the crystalline substance, cholesterin, obtained from biliary calculi, Chevreul proved that these are three distinct substances (21), (22), (23), (24). He was led to this discovery by his careful investigation of some crystals which had formed, after several months, on the surface of a fatty material which had been brought from Rouen for analysis.

In book five Chevreul discussed contemporary views on saponification and proved that acetic acid and carbon dioxide are not produced and that gaseous oxygen is not necessary for the saponification reaction. He also determined the weight of fat which can be saponified by a given weight of potash, *i. e.*, the saponification number of the fat (17), (20). Book six contains a résumé of the researches and their applications, and discusses the properties of the stearates, "margarates" and oleates of sodium and potassium and their solubility in alcohol, acids and alkalis.

In 1825 Chevreul and Gay-Lussac obtained a patent for the production of fatty acids for the manufacture of candles by treating tallow and oils with potash, soda or other alkali and then decomposing the resulting soap with hydrochloric acid. Since the glycerol could be recovered from the aqueous solution, it, too, could be produced on a large scale.

Although the first candles made by the Chevreul-Gay-Lussac method were greasy and unsatisfactory, the engineer, Jules de Cambacérès, improved them by his invention of the plaited wick, and in 1831 M. Adolphe de Milly, one of Chevreul's former students, and M. Motard introduced the use of lime instead of caustic soda and began to manufacture excellent

stearin candles near the Barrière de l'Étoile, which became known as "star, or adamantine" candles. They had been made possible by Chevreul's discovery that the removal of glycerol from fats enormously increases their hardness and illuminating power (21), (25), (26).

To appreciate the importance of this improvement, one must view it through the eyes of Chevreul's contemporaries. "The stearic candle industry," said A. W. von Hofmann, " . . . opens a new era in the history of illumination. In the present generation only the oldest still recall the tallow candle, soft and dripping, disagreeable in color, giving off a sickening odor, requiring constant care as it burned and giving only a dim, smoky flame. Suddenly the tallow candle was replaced by the stearic candle of brilliant whiteness, odorless, hard and sonorous, burning without the slightest aid and with a bright flame. It is your hands which have opened to the grateful world a source of light in no way inferior to that of wax candles, capable of competing with gas light, the use of which was already widespread, and which does not seem menaced by the illumination of the future, by the electric light" (16), (27).

In observing the centenary of Chevreul's discovery on which the stearic candle industry is based, the late Henry E. Armstrong said, "Chevreul was the first to study the 'gas' from which Faraday, a year later, was to isolate benzene . . . To-day Chevreul and Faraday march arm in arm, illuminating the world—the one bearing the candle, the other the electric torch . . . Working as chemists, Chevreul and Faraday between them, practically at the same time, laid the foundations of organic chemistry: Chevreul of its open or paraffinoid, Faraday of its closed or phenoid systems . . . Like Faraday, Chevreul was no mere laboratory worker; he was not only a philosopher but also a systematist and logician of the first order. His scientific writings are all models of clear and simple statement . . . The *bougie stéarique* is no mere illuminant to-day but something at which we can greatly marvel. The chemist can see massed in it wondrously built, tall staircases of atoms, up which the imagination may climb to infinite heights . . . " (15).

In 1824 Chevreul published his great work entitled "General considerations on organic analysis and its applications" (28), the first part of which is devoted to proximate organic analysis and the second to its applications. Like the naturalists, Chevreul used to classify everything into families, genera and species. Among the immediate, or proximate, principles he included the sugar, gum, starch and lignin of a plant; the fibrin and albumin of the cellular tissue of an animal. In the term *species* he included these immediate principles and compounds of two or more of them according to definite proportions. He applied the term *variety* to specimens of a given organic species which differ in secondary crystalline form or other minor properties from the substance considered as typical of the species. By *genus* he meant a collection of organic species which possess one or more important properties in common. He conceived of each specimen of a given species as an aggregation of identical individuals and realized that the true individuals are the compound atoms (molecules).

Berzelius had stated that blood fibrin, albumin and gelatin are partly converted by the action of alcohol and ether into a fatty substance similar to adipocere. Chevreul, realizing that, if alcohol and ether were to be used in proximate organic analysis, this point must be settled, proved quantitatively that the fatty substances contained in alcohol and ether placed in contact with tendons, fibrin, etc., had merely been extracted from those substances. On treating elephant tendons with alcohol, he obtained stearin and olein. When he treated the same tendons with dilute potash, he produced a quantity of stearic, "margaric" and oleic acids equivalent to the stearin and olein previously obtained by means of alcohol. Berzelius later extracted fat from the fibrin of ox blood and acknowledged that Chevreul's statements were correct (29).

Chevreul's book on organic analysis discusses, among other things, the effect of heat and of oxygen on organic compounds; solvents used in organic analysis; saponification as a method of analysis; and the applications of proximate organic analysis to the arts and industries and to medicine, pharmacy, toxicology and the biological sciences.

In the section on pharmacy he wrote: "If in medicine, in a given case, one is in doubt as to the dose of medicaments such as the sulfates of soda and of magnesia, the subphosphate (sousphosphate) of soda, the bitartrate of potassium or of emetic, the composition of which is rigorously defined, there is all the more reason for being so when it is a matter of prescribing such medicaments as extract of opium, cinchona barks, ipecacuanha roots, etc., which contain unknown proportions of the active principles. Proximate organic analysis, which gives the methods of isolating these principles from the foreign substances with which they are united or mixed in the extracts, barks, roots, etc., and which, by defining them as *species* possessed of constant properties, brings them to the condition of the first medicaments of which I spoke, renders eminent services to pharmacology by destroying a cause of uncertainty which the use of many of the most important remedies of the healing art used to present. Physicians, then, should lend full support to researches such as those of MM. Sertürner, Robiquet, Boullay, Gomes, Magendie, Pelletier, etc., to whom we owe the discovery of morphine, of the vesicant principle of cantharides, of picrotoxin, of cinchonine, of quinine, of emetine, of strychnine, of brucine. Researches of this nature are well suited to change the opinions of those who believe with Descartes in the danger of *the remedies of chemistry*" (28).

Chevreul was evidently referring here to F. W. Sertürner's discovery of morphine in 1805–1817; P.-J. Robiquet's analysis of cantharides in 1810;

P.-F.-G. Boullay's discovery of picrotoxin in 1811; B. A. Gomes' memoir on the gray ipecacuanha of Brazil (Lisbon, 1801); the researches of Joseph Pelletier and F. Magendie on ipecacuanha in 1817; and the discovery of strychnine in 1818, of brucine in 1819 and of quinine and of cinchonine in 1821 by Joseph Pelletier and J.-B. Caventou. In his eulogy at the funeral of Robiquet, Chevreul said: "His examination of cantharides informs us of the presence of uric acid in insects which feed on leaves and also of the existence of a principle to which they owe the property of acting as a vesicatory, a discovery which is remarkable in that, by demonstrating as early as 1810 the possibility of extracting the active principle of a complex medicinal substance, it can be considered as the point of departure for numerous researches on this subject . . . "

Chevreul also pointed out in his treatise that, when the medicinal property of a root, bark or extract is due to a combination of several principles and not to any one of them alone, such "active combinations" may be separated from the foreign materials which weaken their essential properties and make correct dosage difficult.

Conservative pharmacists had raised two objections to the use of the pure active principles: (1) it is more difficult to weigh out a few milligrams of the active principle than to weigh several decigrams of the natural product; and (2) the advantages of isolating the active principle are more than counterbalanced by the labor and chemical knowledge required. To the first, Chevreul replied that the active principle could be intimately mixed in known proportion with an absolutely inert constituent; to the second, he asserted that "in the interest of the public, the preparation of remedies should not be very easy, for, the more scientific knowledge it requires, the more pharmacists charged with the sale of medicaments will keep the traffic in them exclusive" (28).

To Chevreul, pharmacy was not merely a heritage from the past but an advancing science of the future. "The most heroic remedies of medicine," said he, "were found only by tests and experiments. If, then, we do not wish to leave entirely to the less enlightened ages the honor of having discovered them, if we think it possible to discover new ones, the torch of the sciences must enlighten us."

At the close of the book he discusses the origin of life. Although he did not believe that the phenomena of life and those of inorganic matter have identical causes, he added that "it must not be concluded that I share the opinion of those scientists who claim to explain the mysteries of organization by one or more forces which they call vital." Even before Wöhler's synthesis of urea had been announced, Chevreul stated that "it would be contrary to the spirit of chemistry to base a classification on the impossibility which has hitherto existed of completely forming an organic compound absolutely identical with one which formed part of an organized being; and according to what we know to-day, there are more reasons for hoping that this formation will be accomplished than there are for believing the contrary" (28).

On September 9, 1824, Chevreul became director of the dye plant at the Gobelin Tapestry Works (30). "On entering the Gobelins," said he, "I found neither barometer nor thermometer nor accurate balances nor platinum ware nor mercury trough nor reagents; the laboratory was a sort of stable or kitchen, paved and damp." He devoted the years from 1828 to 1864 to a study of colors and the technique of dyeing. His first published work on color appeared in 1830 under the title "Memoir on the influence that two colors can have on each other when seen separately" (31). In 1829 and 1830 Chevreul published his lectures on chemistry applied to dyeing (32), and in 1832 he discovered creatine as a normal constituent of muscular tissue (21), (33).

Between the years 1836 and 1862 he read before the Academy of Sciences fourteen memoirs based on his researches on dyes. In these he compared the affinities of various textiles for sulfuric acid solutions by analyzing a known weight of the solution before and after contact with the textile; determined the proportions of water which thoroughly dried textiles absorb at different temperatures; studied the separate and combined effects of water, light, atmospheric agents and hydrogen on textiles dyed with weld, yellow wood, archil, Brazilwood, Campeche wood, madder, turmeric, cochineal, annatto, safflower, sulfoindigotic acid and Prussian blue; investigated the methods of removing grease and sulfur from wool, and the chemical composition of wool fat; showed the effect of the mordant on the tone of the color and on its stability in sunlight and discussed the theory of bleaching and the preservation of colors. He investigated the effect of temperature on the value of the mordants used; studied the effect of natural and artificial contaminants of the fabrics on the beauty and durability of the color and compared distilled water, Seine River water, Parisian well water and artificially prepared waters as to their suitability for use in the dye baths (34).

In 1828, soon after his work on fats and organic analysis had opened up two great fields of research, Chevreul broke ground in a third new field, the psychology of color, and in 1839 he published his great work on "The principles of harmony and contrast of colors and their application to the arts" (35). He showed that the chemical composition of the colors had nothing to do with the effects of their simultaneous contrasts; distinguished between simultaneous, successive and mixed contrast of colors; showed how to imitate colored objects with the coloring materials in a state of very small division and how to produce given effects with colored threads and applied his results to the production of Gobelin tapestries, Beauvais furniture tapestries and Savonnerie carpets, to color printing on cloth and paper, to horticulture and to the making of maps, mosaics, glass windows, engravings and clothing. He himself stated that his work on contrast of colors was just

"as experimental and as exact" as his previous treatises on fatty bodies and organic analysis (35).

Before he began these researches, the intermediate shades were produced by a process called *rabattage*. To decrease the intensity of a bright color, the dyers plunged the dyed fabric into a dilute solution of a ferrous salt mixed with an astringent. The iron salt then imparted a gray tone to the color, but, when the dyed textiles were exposed to the air, the iron was oxidized, and the gray shades were changed to brown or black. Thus the entire fabric, including even the portions which were intended to be brightly colored, acquired a dingy appearance.

"In searching for the causes of the complaints raised against the quality of certain colors prepared in the Gobelin dye-works," said Chevreul, "I soon convinced myself that if the complaints regarding the instability of the light blues and violets, of the grays and of the browns were well founded, there were others, especially those concerning the lack of vigor of the blacks used to make the shadows in blue and violet draperies, which were unwarranted; for after having procured woolens dyed black in the most famous plants in France and abroad, and after having recognized that they were not at all superior to those dyed at the Gobelins, I saw that the lack of strength complained of in the blacks was caused by the color placed next to them and was due to the phenomenon of contrast of colors; it then became clear to me that, in order to fulfil the duties of director of dyeing, I had two entirely different subjects to treat: the first was the contrast of colors, broadly considered, both in its scientific and its practical aspects; the second concerned the chemical part of dyeing. These, indeed, have been the two centers to which all my researches for ten years have converged" (30), (35). These researches made it possible for the Gobelin plant to produce effects hitherto unknown and to do it with a limited number of carefully chosen colors of well-established permanence.

Feeling the need for a definite standard with which colors could be compared, Chevreul constructed a chromatic circle, using as his fundamental points of comparison certain rays of red, yellow and blue, each of which was marked by a well-defined Fraunhofer line. He placed these three colors at equidistant points on the circle and interpolated twenty-three definite color mixtures in each of the three intervening sectors. In addition to this "normal" circle, he prepared eight others, in which the colors were toned down with definite known proportions of black. He thought that official standards of color ought to be preserved like standards of weights and measures, but realized the difficulty of obtaining enough dyes which would be sufficiently permanent. In his lectures at the Gobelins, he used to construct a mammoth circle of skeins of worsted on the floor of the great exhibition hall (36).

In 1854 he published an exposé of the divining-rod and other psychic and spiritualistic phenomena which were then creating a furor on both sides of the Atlantic (37). Professor Joseph Jastrow has recently published an interesting account of these psychological researches (38).

Chevreul also made notable contributions to the history of chemistry, including an "Histoire desconnaissances chimiques," and a more extensive work "Résumé d'une histoire de la matière depuis les philosophes grecs jusqu'à Lavoisier inclusivement," only a portion of which was published (39), (40). Mme. Metzger has emphasized the fact that these works are well worthy of study by historians of chemistry (41).

When he was about ninety years old Chevreul made a detailed psychological study of the changes which accompany old age (42). In 1883, at the suggestion of J.-B.-A. Dumas, Chevreul, then ninety-seven years old, was appointed "director of advanced studies for the observation and analysis of the colors of dyes" (30). Chevreul had been publishing scientific memoirs for twenty-nine years before the *Comptes rendus* was founded, yet among its great volumes from the years 1836 to 1888, inclusive, there are scarcely any which do not contain one or more of his articles or critical discussions or notices of his appointment to important committees.

Since B.-B. de Fontenelle had died a month before his one-hundredth birthday, and since Chevreul's birthday comes in the summer, when most scientists are away from Paris, the observance of the Chevreul centenary began three months ahead of time. On May 17, 1886, the Academy of Sciences had a ceremony in his honor, and on August 30th and 31st the Society of Agriculture paid homage to him. According to tradition, Chevreul had been elected president of this society every alternate year, and he had fulfilled these duties "with a punctuality one would never expect from a centenarian" (2). When he arrived at two o'clock, he was greeted by the eleven presidents of the sections of the society, each carrying a large bouquet of roses.

The third celebration was that of the city of Paris and the Museum of Natural History, aided by the press. An exhibition of industrial products which had been improved through Chevreul's researches, which had been suggested, had to be abandoned for lack of space to display all the materials which were submitted (36). When the statue of Chevreul was unveiled, the entire Museum was decorated with red velvet, flowers and the finest tapestries, ancient and modern, which had ever been made at the Gobelin plant, and more than two thousand delegates from learned societies, schools, museums and workshops came marching in with banners.

The informal dinner at six-thirty was illuminated by thousands of candles presented by the stearin manufacturers of Paris. When the clock at the Hôtel de Ville struck eight, M. Janssen arose and announced that that very moment marked the centenary of Chevreul's birth. At the request of M. Goblet, Minister of Public Instruction, Chevreul, who had never before indulged in wine, joined the guests in the toast "Vive la France!" An hour later,

wearied by the long ceremonies, he asked permission to retire, and the festivities were concluded with a torchlight procession from the Hôtel de Ville to the Place de la République and gala performances at three theaters.

After the centenary, Chevreul lived very quietly and drove daily to watch the construction of the Eiffel Tower. M. Gaston Tissandier, the editor of *La Nature*, has left us the following picture of Chevreul the centenarian: "M. Chevreul is tall of stature and is, even to-day, slender and erect. Elegant of manner, of incomparable affability, he rarely greets you without a smile on his lips. His head is wonderfully expressive, the forehead large and powerful, covered with white hair. A few years ago he still used to attend the winter ball at the Elysée, and we recall seeing him there at midnight, fresh and smiling, surrounded by ladies, whom he was gallantly entertaining, with exquisite and charming grace" (2). To a new assistant he once said, "You must have courage to accept a position as my préparateur; I have already killed off four of them" (2).

The monograph on the national manufactures of France, which was published in 1889, shortly before Chevreul's death, states that "Thanks to the resolute vigor of his intellect, accumulating discovery after discovery, M. Chevreul has pursued these great researches at the Gobelins up to the present. The famous scientist whose centenary all Paris celebrated two years ago still discharges in an honorary capacity the duties with which he was entrusted in 1824 . . . " (30). Only a few days before his death, Chevreul suffered the loss of his only son, Henri, a bibliophile like himself. The introduction to the "Law of simultaneous contrast of colors" was written by M. Henri Chevreul, and he had also intended to prepare a preface for the reprint of the "Chemical researches on fatty bodies" which was issued in 1889 (17).

The ode by Emile Guiard, which the actor Albert Lambert read at the Odéon at the time of the centenary, eloquently acknowledges society's indebtedness to Michel-Eugène Chevreul:

"Prends la vie de ces gens à la vie inféconde,
Qui ne savent marquer leur passage en ce monde
Que par leur instinct destructeur;
Prends leurs jours consacrés à la haine, à l'envie,
Prends leurs jours et fais-en une éternelle vie
Pour les Chevreul et les Pasteur."

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Book Reviews

Industrial Solvents, by IBERT MELLAN, M.Sc. Reinhold Publishing Company, 330 West 42nd St., New York, N. Y., 1939. 480 pages, 291 figures. Price, $11.00.

Beginning with a brief discussion of theories and facts in connection with solvent action, vapor pressure and evaporation, viscosity, plasticity, inflammability and toxicity, the author presents extensive data concerning the common as well as the lesser known solvents. Classification of solvents is made according to their chemical constitution, *i. e.*, hydrocarbons and hydrogenated derivatives, halogenated hydrocarbons, alcohols, aldehydes, acids, ketones, ethers, esters; the properties, uses and other technical data are given for individual solvents under the foregoing classification. Inclusion of 126 tables and 291 charts, graphs and figures showing the properties of solvents and mixtures of solvents provides for a ready comparison of these substances under varying conditions. A chapter on plasticizers, giving their properties and uses, is also presented. The last of the 17 chapters of the book deals with methods of graphical expression and interpretation, particularly as these apply to the subject of solvents. Abundant literature references are given at the close of each chapter. Chemists and others who are concerned with solvents will find a wealth of information which may well make the book indispensable to them.—ARTHUR OSOL.